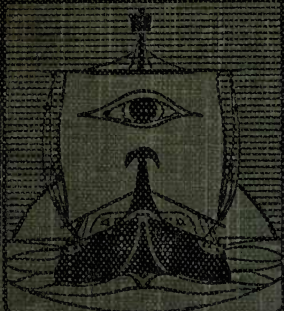


LIVERPOOL SCHOOL
OF TROPICAL
MEDICINE □ □



THE
PRACTICAL
STUDY
OF
MALARIA
AND
BLOOD PARASITES

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THE PRACTICAL STUDY
OF MALARIA

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AND OTHER BLOOD PARASITES

BY

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MEMBERS OF THE ROYAL SOCIETY'S COMMISSION ON MALARIA
IN AFRICA AND INDIA, 1898-1902

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PREFACE TO FIRST EDITION

In the authors' experience many medical men in the tropics are only deterred from undertaking researches in tropical diseases by the impossibility of obtaining the necessary knowledge of methods apart from personal instruction in some laboratory. Numerous works on technique exist, they are, however, more adapted for work in a laboratory than for the conditions under which the average practitioner in the tropics must be prepared to conduct his researches. As a result of an experience of several years, during our work on the Royal Society's Commission on Malaria, of the difficulties that Indian and Colonial medical officers experience in making the first start in what must often be work of the greatest interest to themselves and the utmost value to science, we have deemed it wise to give instead of full and elaborate technique, as usually given, only that which we have found the best, the simplest, and the most generally useful. In reality, the necessary methods required to undertake research of the highest value in Malaria are very simple, yet most of these cannot be found in books, and they are with considerable difficulty learnt except by the personal direction of those who are familiar with the small details which go to make success.

In the present handbook we propose to give the essentially practical methods, by which those not familiar with laboratory methods may, under their own microscopes, follow all the most recent work on

Malaria, and eventually be in a position themselves to add new facts to our knowledge of this important disease.

For instance, with very little apparatus it is possible to undertake many most important researches, *e.g.*, to work out the rationale of infection in any station or cantonment; the form of the parasite present; the percentage of adults and children infected; the species of *Anopheline*; where each species is found and where it breeds; the percentage of each species carrying sporozoites and zygotes.

In fact nearly the whole technique of Malaria can be conducted with a microscope, a few slides and coverglasses, a needle, a stain, some tubes, pins, and cardboard. (*Vide* Appendix).

While our original intention was to write a practical guide to Malarial Study solely, yet the opportunities for research on other blood parasites are so numerous in the tropics, that we have thought it to be of practical value to add short supplementary chapters on other Haematozoa and on the Trypanosomidae, etc.

November, 1903

PREFACE TO THE THIRD EDITION

In the present edition we have to add many new blood parasites. We have thought it best to describe most fully those which are of pathogenic importance, and which cause well-known diseases either in man or animals, so that in the present edition the pathogenic Trypanosomes, Haemamoebidae and Spirochaetes are described more at length than hitherto. A knowledge of ticks has become increasingly important, and the chapters dealing with them have been rewritten and much extended. In order to make room for this new matter we have omitted other matter of less importance, *e.g.*, in our consideration of mosquitoes we have found it impossible, and indeed unnecessary, to consider them as a whole, but have endeavoured to describe concisely the Anophelinae only. We have also thought it best to omit the chapter dealing with Filaria. We include, on the other hand, short descriptions of yellow fever and a new unclassified parasite of man occurring in Panama, namely, *Histoplasma capsulata*.

February, 1908

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The Practical Study of Malaria

Chapter I

THE NORMAL CONSTITUENTS OF THE BLOOD

NORMAL blood should be carefully studied in fresh and stained specimens.

1. *Red Cells* (or normocytes).— $6-9\mu$. In fresh films they show a very faint central pale area. The colour on staining with Romanowsky (p. 21) varies with the amount of washing; generally it is greenish or bluish, or faint eosin; with Leishman, Giemsa, etc., a deeper eosin colour.

2. *Platelets*.— $2-4\mu$. Vary much in size and shape. They frequently occur in masses in wet films and if examined an hour or so after formation these masses exude colourless drops of secretion best seen in 'vital staining' specimens (Ross). They stain bright crimson with Romanowsky and lie in clumps of from six to fifty. They do not shew the red, white, and blue of a well-stained parasite but stain diffusely crimson or blotchy violet (Fig. 2).

3. *Blood dust* (or *Haemaconiae*).—Refractive granules, smaller than micrococci, at most 1μ in diameter. They shew active motion (Brownian) in fresh films. They are probably granules escaped from leucocytes.

4. *Fibrin*.—Appears as a meshwork of very delicate fibrils in the thicker parts of the film.

ABNORMAL CELLS AND CONDITIONS

Normoblast.—A nucleated red cell about the size of a normocyte (Fig. 1). Two forms occur (*a*) young forms with the nucleus having its chromatin radially arranged (*radkern*) staining more deeply than that of any leucocyte and with a narrow rim of protoplasm. (*b*) Older forms with dense nucleus and with broader protoplasm. The protoplasm may shew polychromasia (*vide* below) or basophilia, or both together.

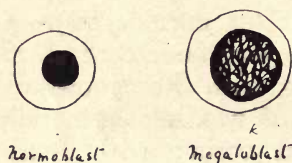


Fig. 1

Megaloblast.—A nucleated red cell of abnormal size (Fig. 1). The network of the nucleus is more delicate and stains less deeply than that of any leucocyte. The protoplasm may be very polychromatic so that its resemblance to a leucocyte is then considerable. Basophilia also may be present.

Pyknosis.—The condition of the nucleus in which instead of shewing a network, it appears quite structureless and stains more deeply than usual. Probably it is an indication of degeneration. This condition may be seen in the two previous erythroblasts.

Karyorhexis.—The condition in which the pyknotic nucleus of nucleated red cells has broken up into

several portions. Not uncommonly the protoplasm at the same time shews basophil granulation. Such forms may be mistaken for polynuclear leucocytes. This condition occurs in severe anaemias.

These two conditions are not at all common in the blood in malaria or even blackwater fever, but are well shewn in marrow films of blackwater fever.

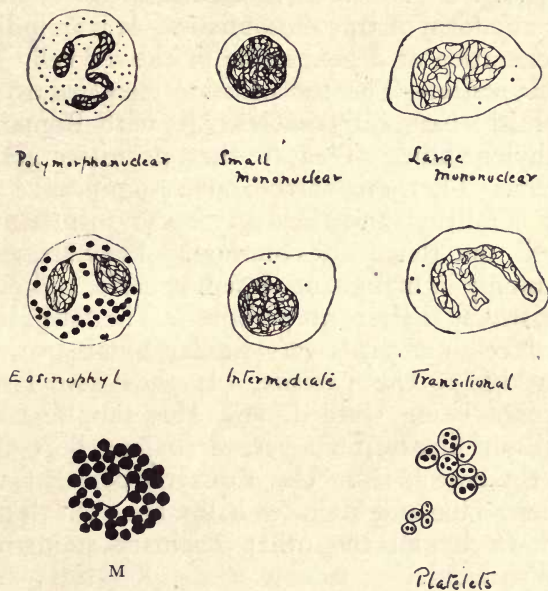


Fig. 2. Normal constituents of Blood:—

M = Mast Leucocyte

Small Mononuclear = Lymphocyte

Intermediate = Mature Lymphocyte

Free nuclei.—Of nucleated red cells are generally structureless and stain very deeply (pyknotic). Sometimes they are surrounded by a trace of protoplasm.

Polychromasia.—The condition of normal and nucleated red cells in which the protoplasm, which normally is acidophil (*e.g.*, stains with eosin), becomes to a greater or less extent basophil, *i.e.*, stains more or less deeply with a basic stain, *e.g.*, methylene blue, so that if stained with eosin and methylene blue the colour of the red cell would be uniformly purplish. Seen in anaemia, *e.g.*, of blackwater fever, and is very common in almost any form of trypanosomiasis. It is an indication of regeneration or degeneration of the red cell or both.

Basophilia.—The red cells are *sprinkled* over with fine (bluish) basophil granules, *e.g.*, with Romanowsky or methylene blue. They are the 'primitive granules' of Plehn. They are of the same significance as the former condition and they may occur together in the same cell, but this is not common. They are seen not uncommonly in malignant tertian malaria, and occur in cells in which there is no parasite. The 'stippling' of infected cells (p. 32) is very similar but is in this case directly due to the parasite. If these two last conditions are being studied, and this should be done especially in blackwater fever, methylene blue alone is one of the best stains. Use a quarter per cent. watery methylene blue and stain for half a minute. They are also shewn by all the other ordinary stains except Ehrlich's triacid.

NORMAL LEUCOCYTES

Polymorphonuclear leucocyte.—10-12 μ .^{*} Also called the neutrophil leucocyte because the granules stain (a

* It is very important to remember that the size of leucocytes and the depth of colour of their nuclei vary according as the leucocytes have been much or not at all flattened in the making of the films.

red-violet colour, ϵ granulation) with the neutral constituent of Ehrlich's triacid stain. The nucleus is convoluted, horse-shoe or S-shaped, staining deeply. The granules are fine and stain reddish with Romanowsky. These cells are markedly amoeboid and = mikrophages; but malarial pigment is very uncommon in them (Fig. 2).

Eosinophil Leucocyte.—12-15 μ . The nucleus is polymorphic in type but very frequently consists of two lobes joined by a strand (*pince-nez*) staining less deeply than the previous form. Characteristic are the large granules (α or oxyphil granulation), which stain coppery red with triacid, Jenner, Leishman and Giemsa, but faintly only or not at all with ordinary Romanowsky, unless the blue is well washed out (Fig. 2).

Mast Leucocyte (or basophil cell).—About 10 μ . The nucleus is of an irregular characteristic polygonal shape, and stains very feebly. Characteristic also are the basophil (γ) granules, which are even larger than eosinophil granules. They are soluble in water, so that to shew them properly an alcoholic stain is necessary, *e.g.*, Jenner's. Triacid does not shew them. Romanowsky, Giemsa, and Leishman do not shew them well. With absolutely pure methylene blue they stain blue, but methylene blue nearly always contains a trace of 'azure' and they stain instead with this derivative, *i.e.*, they stain violet. This is spoken of as staining metachromatically; and this property of staining, not with a pure stain but with its derivative, is characteristic of mast granules (Fig. 2).

Lymphocyte.—Two forms occur (*a*) young forms 6-9 μ , *i.e.*, about the size of a red cell, forming the majority. These have a round or oval nucleus staining deeply, and the rim of protoplasm is quite narrow and deeply basophil. (*b*) Old forms about 12 μ . The

nucleus is frequently eccentric and may be slightly indented, and does not stain so deeply as the young nucleus. The protoplasm is fairly wide and often shews some reddish granules with Romanowsky. These forms are not always easily distinguished from large mononuclears, the greater size of the latter and the character of the nucleus are the main distinctive features. These old forms constitute one of the cells known as makrophages. They sometimes contain malaria pigment (Fig. 2).

Many of the lymphocytes in fresh films shew a spot at the edge of the cell (Manson's spot). The spot looks dark in one focus, light in another, and should not be mistaken for a pigment granule.



Fig. 3. *Myelocytes.* Left, *Eosinophil*; right, *Neutrophil*

Large Mononuclear Leucocyte.—10-20 μ , the largest leucocyte in the blood. It has a characteristic irregular much-indented nucleus which stains feebly, quite distinct from that of the old lymphocyte. The protoplasm is broad and may shew a few azure granules with Romanowsky. It also is a makrophage, and is the typical pigmented leucocyte of malarial blood (Fig. 2).

Transitional.—About the same size as large mononuclears, and the nucleus is of the same general nature; but the lobed indented character is much further developed, being trident or S-shaped. They are not uncommon in malarial blood and are counted with the large mononuclears (Fig. 2).

PATHOLOGICAL LEUCOCYTES

1. *Neutrophil Myelocyte*.—15-20 μ . A mononuclear leucocyte with numerous neutrophil (ϵ) granules. The nucleus is round or indented and stains feebly. Very common in myeloid leukaemia; it is also found in the blood when there is a marked leucocytosis or in pronounced anaemias, *e.g.*, of malaria (Fig. 3).

2. *Eosinophil Myelocyte*.—As the former but with eosinophil (α) granulation. Found mainly in myeloid leukaemia and occasionally when the blood shews well-marked eosinophilia (Fig. 3).

3. *Mast Myelocyte*.—As the former but with basophil (γ) granules which stain, however, with watery stains. In myeloid leukaemia.

4. *Large Lymphocyte*.—15-20 μ , *i.e.*, slightly larger than large mononuclears. The nucleus is *very large* and *round*, stains feebly, but has one or two distinct nucleoli. There is a *scanty* rim of protoplasm shewing a fine basophil network. They are distinguished from large mononuclears by the round nucleus, by the nucleoli and by the narrow rim of protoplasm; from the mature or old variety of normal lymphocyte by the fact that the nucleus is quite round and not oval or long and eccentric, by the difference in staining, and by the scanty, not broad, rim of protoplasm. Normally they occur as tissue cells in lymphatic glands and in the lymph follicles of the spleen. They occur in the blood in lymphatic leukaemia and allied conditions (Fig. 4).

5. *Myeloblast* = lymphoid marrow cells.—10-20 μ . The nucleus is round or oval and stains deeply (not faintly as in the large mononuclear and older lymphocytes) and a dirty grey-blue with triacid (not bluish green as the lymphocytes). The protoplasm is narrow and deeply basophil, more so than that of the large

variety of lymphocyte, and Romanowsky shews no granules.

These cells require very careful staining for their distinction. Special stains, *e.g.*, pyronin methyl-green, shew three or four nucleoli stained reddish, whereas other leucocytes shew only one or two. They are perhaps most readily recognised by the fact that all stages from these forms to neutrophil myelocytes occur together in the blood. They occur in abundance in myeloid leukaemia and are found also in other anaemias. (Fig. 4).

Plasma Cell.—= inflammatory leucocyte or phlogocyte. They are large cells, $10-20\mu$, generally $10-15\mu$.



Fig. 4. (1) Large Lymphocyte, (2) Myeloblast, (3) Plasma Cell

Nucleus, round or oval, usually eccentric, relatively small, staining a dirty grey-blue or violet with triacid. No radial arrangement of chromatin, which resembles that of a myelocyte. Characteristic is the intense basophilia of the protoplasm (staining reddish brown with triacid), broad margin and very distinct highly characteristic honey-combed structure. With methylene blue the protoplasm stains deeper than the nucleus. They occur in leucocytosis and severe anaemia (Fig. 4).

In the tissues in sleeping sickness (p. 340) we have 'Marschalko's plasma cell.' In this the chromatin of

the nucleus is arranged in a spoke-like fashion ('rad-kern'). The nucleus is at one side of the cell and is separated from the cytoplasm by a halo.

The leucocytes may be studied and counted in films stained with Romanowsky, but for many purposes it is advantageous to use one or more of the following stains. For studying the character of the nuclei, haematein is the best; for the granules, Ehrlich's triacid; and for mast granules, Jenner.

1. *Ehrlich's Triacid* (*vide* p. 408).—Fix with heat (p. 20). Without shaking the bottle pipette off enough to cover the blood film. Stain for five minutes. Wash in water. The neutrophil granules (ϵ) are reddish violet; the eosinophil (α) are coppery red; the red cells, orange; the mast granules and basophilia of the red cell are not shown. The stain is best made by one's self.

2. *Eosin and Methylene Blue*.—Fix with heat, stain with eosin (p. 408) three minutes. Wash (and dry). Stain with methylene blue (p. 408) two parts and eosin one part, freshly mixed, for half a minute. Wash. Dry.

3. *Eosin and haematein*.—Fix the film. Stain with eosin three minutes. Wash, stain with haematein (p. 408) half to one minute. Haematein is especially good for studying the differences in the nuclei.

4. *Jenner's Stain* is the precipitate resulting from the action of a watery solution of eosin on a watery solution of pure unripened methylene blue; dissolved in methyl alcohol (Tabloid 0.05 gramme; methyl alcohol 10 c.c.).

To stain.—Fixing is unnecessary. Place solution on film, covering so as to avoid evaporation. Stain two to three minutes. Add about twice as much H_2O to the slide. Mix. Stain five to ten minutes. Especially adapted for the mast granules.

TO MAKE A DIFFERENTIAL COUNT OF THE LEUCOCYTES

Large films are necessary, especially in malaria where, during the apyretic period, there is a distinct diminution in the *total* number of the white cells. It is important, in making films for leucocyte counting, that the margins and terminal points of the film be regular, and so in a convenient position for examination (Fig. 5). The margin of the film is focussed and passed beneath the oil immersion lens. By passing along the whole of the *margin* of the film, the great majority of the leucocytes in the film are seen. In order to obtain accurate results, one thousand leucocytes should be counted, but a count of three or four hundred is generally sufficient for diagnostic purposes. Counts of a smaller number of leucocytes are valueless, as too great variations will occur.

As a leucocyte is seen, it is marked under the heading, large mononuclear, transitional, polynuclear, eosinophil, lymphocyte, as the case may be. As many as ten to twenty or more are noted mentally before making each record in its column.

NORMAL LEUCOCYTE VALUES

Polymorphonuclear leucocytes,	65-70 per cent.
Large mononuclear and transitional	„ 3-5 „
Small mononuclear or lymphocytes	„ 20-25 „
Eosinophil	„ 2-4
Mast or basophil	„ 0.5 „

TO COUNT THE RED CELLS

*The Thoma-Zeiss Pipette.**—For counting the red cells has a narrower calibre than that used for counting white cells and the top of the bulb is marked 101. The stem is equal to one part, consequently the bulb is equal to one hundred parts, and when one part of blood and diluting fluid fill the bulb, the dilution of the former one part is one hundred times. The glass ball in the bulb is simply to aid the mixing process.

Diluting Fluid.—Blood cannot be counted without dilution as it is too 'thick.' In an emergency 0.9 per cent. salt solution may be used but preferably Hayem's fluid, viz. :—

Mercuric chloride	1 part
Sodium chloride	2 parts
Sodium sulphate	10 „
Water	400 „

Counting Chamber.—The central disc has a number of minute squares (400) ruled upon it. The side of each smallest square is $\frac{1}{20}$ mm. Therefore, the area of each square is $\frac{1}{400}$ mm.², as marked on the slide. Further, when the cover glass is in position the depth

* TO CLEAN PIPETTES

For any accuracy of observation the pipettes should be scrupulously clean. There should not be the slightest tendency for the glass ball to stick to the sides. After a count has been made, the rubber tube is removed and the contents ejected by blowing from the pointed end.

1. Suck up dilute acetic acid so that all traces of stain are removed.
2. Suck up several lots of clean water to remove the acid.
3. Then absolute alcohol two to three times to remove the water.
4. Then ether two to three times to remove the alcohol.
5. Finally, blow hot air through with a syringe, the glass barrel of which may be heated in a flame (or simply suck air through).
6. Occasionally it is necessary to clean the bulb by digestion. The pepsin or trypsin can be got in tabloid form.

of fluid lying over the squares is $\frac{1}{10}$ mm. (= 0.1 as marked on the slide). Therefore, the volume of fluid lying over each square is $\frac{1}{100} \times \frac{1}{10} = \frac{1}{1000}$ mm.³. Suppose, now, that on counting we find ten red cells on a square. Then in $\frac{1}{1000}$ mm.³ there are ten red cells. Therefore in 1 mm.³ there are $10 \times 4000 = 40,000$ red cells. But the blood has been diluted 100 times. Therefore, normally, there would be $40,000 \times 100 = 4,000,000$ in 1 mm.³.

In practice it is not sufficient to count one square but it is necessary to count many. If then we add up all the red cells (*e.g.* 1000) counted and divide by the number of squares counted, we get the number for one square. So that we get this formula for the number of corpuscles per mm.³

$$\frac{4000 \times \text{dilution} \times \text{total number of red cells counted}}{\text{Number of squares counted.}}$$

The normal average values are for man 5,000,000 and for woman 4,500,000.

Orientation Lines.—Every fifth row of squares has a line drawn through its middle dividing it into two oblongs. These lines are simply to prevent the eye losing itself among so many small squares, and for purposes of counting or calculation are disregarded.

Procedure.—1. The pipette must be quite dry and the glass ball move quite freely.

2. Prick the finger; the blood must flow freely. Pressure on the finger must not be used as plasma is squeezed out of the tissues.

3. Place the tube in the mouth and hold the pipette so that the scale is completely in view.

4. Suck up blood exactly half-way, *i.e.*, 0.5 for bloods fairly normal, but to 1 for anaemic bloods.

5. Wipe off adherent blood, and placing the point

below the surface of the fluid suck up carefully to the mark 101.

6. Rotate thoroughly between finger and thumb for some minutes.

7. Blow out the stem contents which are only diluting fluid and then rapidly put a small drop on the centre of the disc.

8. Breathe on the cover glass and lower it rapidly into position with a needle, so as to avoid bubbles.

The fluid must cover the disc completely, and there should be no air bubbles. If not filled properly, rotate the pipette thoroughly before trying a second drop.

9. When Newton's rings (*i.e.*, the play of colours seen on a film of tar, etc.) are seen between cover glass and slide, then, and not till then, is the former in its correct position and counting can be proceeded with.

10. Make certain that the lines of the squares can be focussed with the lens used, *e.g.* $\frac{1}{4}$ Leitz. Generally it is necessary to use the thin cover glass supplied and not the thick one. Take care that the diaphragm of the microscope is as nearly closed as possible.

11. Examine with a $\frac{1}{2}$ -inch lens to see that the red cells are evenly distributed over the squares. If this is not so the count is not reliable, and errors in mixing or in filling the chamber have probably arisen.

12. In case of red cells overlapping the sides of a square, even in the slightest degree, count only those on two adjoining sides.

13. Count a thousand red cells.

TO COUNT THE WHITE CELLS

The Pipette for Leucocytes.—Has a stem of fairly wide calibre. Above the bulb is the mark 11. The

stem contains one part. The dilution of the fluid in the bulb is therefore ten times (*vide supra*). The object is to use blood as little diluted as possible, as leucocytes are comparatively few in number.

The Diluting Fluid.—About one-half per cent. acetic acid is used in order to dissolve the obscuring red cells. To this is added a little filtered stain, so that the nuclei of the leucocytes are coloured.

The Counting Chamber.—If we take the normal value for leucocytes to be 10,000 per mm.³; then, on 400 squares (*i.e.*, the whole of the ruled area) there would be $10,000 \times \frac{400}{4000} = 1000$. But the blood is diluted ten times so that 100 would be the possible maximum. Now this number is too small to give an accurate result, so that it is necessary to use a chamber which has a set of extra squares ruled, *e.g.*, Türk's leucocyte counter. By the use of this it is theoretically possible to count 900 leucocytes.

Calculation.—The number of leucocytes per mm.³

$$= \frac{4,000 \times \text{total number leucocytes counted} \times \text{the dilution}}{\text{Number of squares counted.}}$$

The normal value for leucocytes is about 10,000.

Procedure.—1. Pour out the diluting fluid into a watch glass.

2. Suck up the blood, which must be plentiful, to mark 1, holding the pipette nearly horizontal, as otherwise the blood will flow out. This tendency may be checked by plugging the rubber tube with cotton-wool.

3. Wipe off blood from outside of the point.

4. Place the finger on the point and then place the point below the surface of the diluting fluid.

5. Now suck and, *while still sucking*, remove the

finger from the tip; the diluting fluid then enters. Keep the pipette nearly horizontal. Fill to the mark 11.

6. The rest is the same as for the red cells.

7. Count a thousand leucocytes, if possible.

The use of a special counting chamber may, however, be obviated by the method of counting fields.

1. Draw the tube of the microscope out so that the diameter of the microscope field is *exactly* equal in length to that of eight squares of the counter. Mark this point on the tube for future use. The area of the field is then almost equal to that of fifty squares.

2. Now count the leucocytes in say eighty fields. This would give 4,000 squares (and normally a possible total of 1,000 leucocytes).

3. The calculation is the same as before.

THE ESTIMATION OF THE HAEMOGLOBIN

Tallqvist.—The blood drop is absorbed by a strip of blotting paper and compared directly with a series of coloured standards. Clinically useful and accurate to within 5 or 10 per cent.

Gowers.—The standard of comparison is here a tube of picrocarmine gelatine equivalent in colour to one per cent. solution of normal blood. The height of this solution is the same as that marked 100 in the second tube, and is the height occupied by 2 c.c. of fluid. The blood pipette holds 20 mm.³ Hence 20 mm.³ in 2,000 mm.³ (2 c.c.) = 1 in a 100, *i.e.*, one per cent. solution of normal blood. If now the colours correspond the blood examined is normal. If less water is required the blood is so much per cent. below the normal; the readings being made directly. Daylight is used.

The standards fade in the tropics and must be returned for graduation.

Procedure.—1. Place a little water in the graduated tube.

2. Fill the pipette carefully, holding it almost horizontal, and wipe off any blood on the outside.

3. Blow out into the tube. Now wash out the pipette thoroughly by sucking up and blowing out successive lots of water from the rubber pipette supplied.

4. Add water by successive amounts of five scale divisions and stir thoroughly with a piece of wire or probe.

Sahli.—Is constructed on the same principle as the Gowers, except that in this case the standard is one of acid haematin. The blood to be examined must therefore be treated with about ten times its volume of one-third per cent. hydrochloric acid; then fill up with water.

It is a very accurate instrument. *Gently* shake the standard when a sediment occurs.

Fleischl-Miescher.—Fully described in the book accompanying each instrument. It is very accurate but expensive.

For choice use a Talqvist and Sahli.

COLOUR INDEX

When the haemoglobin is 100 per cent. and the red cells five million (*i.e.*, with the ratio $\frac{100 \text{ Hgb}}{5,000,000 \text{ R.b.c}}$) the colour index is said to be 1.

If this ratio were equal to 1 the colour index would then be 50,000. So that to find the colour index, after counting the red cells and estimating the

haemoglobin, we simply have to multiply the ratio $\frac{\text{Hgb}}{\text{R.b.c}}$ by 50,000.

Example 1.—Let the red cells be 525,000 and the Hgb 17 per cent.; what is the colour index ?

It is $\frac{17}{525,000} \times 50,000 = 1.6$, as in pernicious or *Dibothriocephalus latus* anaemia.

Example 2.—Let the red cells be 4,000,000 and Hgb 25 per cent., what is the colour index ?

It is $\frac{25}{4,000,000} \times 50,000 = .31$, as in chlorosis.

Chapter II

TO PREPARE BLOOD FILMS

To Clean Slides.—Slides should be dipped in water and rubbed dry and clean with a soft cloth, *e.g.*, a clean handkerchief. If a perfectly clean slide is required, heat it 'red hot' over a flame; in this way grease is completely removed.

Before proceeding to take specimens of blood, the prepared slides may be placed in a small pocket slide box or wrapped in a sheet of clean note paper.

To Clean the Patient's Finger.—If the finger of one's subject is obviously dirty, and especially if damp with sweat, the finger should be wiped with a cloth. If considered necessary, precautions may be taken to avoid all skin contaminations by the use of soap and water, alcohol, and ether, but in ordinary examinations for malarial parasites this is quite unnecessary.

To make Dry Films.—The simplest and by far the best way of making films is by the use of no other apparatus than—

1. A straight surgical needle about two inches in length with the eye cut off.
2. Clean glass slides.

When the drop of blood reaches the size of the head of a *small* pin, touch it with the slide about one-third inch from the far end. Now lay the shaft of the needle across the drop of blood. After waiting about a second, that is until the drop spreads between the slide and the needle; the needle is evenly and not too

quickly carried to the right along the whole length of the slide (Fig. 5). Immediately the film is made it should be waved to and fro until it is seen to be quite

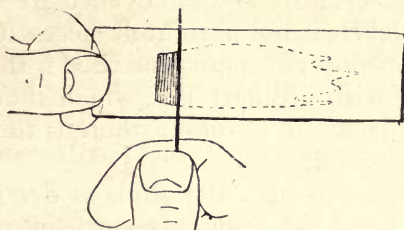


Fig. 5. *Authors' method of making blood films*

dry. The quicker the film dries the more perfectly preserved will be the red cells of the blood.

THE CHARACTERS OF A GOOD FILM

1. As a film is needed for the detection of minute forms of the parasite within the red cell, the film must be uniform and as thin as possible.

2. Films should be made so that, if desired, the leucocytes may be differentially counted. A little practice will enable one to make films with the upper and lower edges more or less parallel with the edges of the slide, and terminating in a pointed manner about half an inch from the right hand end of the slide.

3. In the case of very anaemic bloods, *e.g.*, those of 'malarial cachexia,' difficulty will arise from the film being too thin. The needle in this case must be carried very loosely and rapidly along the slide and a thicker film thus made. When blood with difficulty adheres to the slide, good evidence of extreme anaemia is obtained.

TO LABEL FILMS

Films should always be labelled as soon as possible, otherwise uncertainty and annoyance are sure to arise. The use of labels is not absolutely necessary.

1. The most convenient method is that of writing on the slide with ordinary ink, which should be quite dry before placing in alcohol ; there is then no fear of the ink coming off.

2. After making a dry film, as described above, the name, date, and other necessary information, are scratched on the film with the head or point of the needle. (Powell).

TO FIX FILMS

Films must always be fixed, but this may be omitted where the stain also contains the fixative, *e.g.*, in the Leishman and Jenner stains.

1. *Absolute alcohol*.—This is the most convenient fixative. It is best kept in a glass-stoppered cylindrical jar about two inches in diameter. Methylated spirit can be used instead. Fix for three minutes or longer.

2. *Heat*.—For the study of leucocyte granules this mode is indispensable. Place a crystal of urea (M. Pt. 131° C.) on the film to act as an indicator. Heat over a flame until the crystal just fuses. Allow to cool and remove the urea. The slide is then fixed. An alcoholic stain must still be used, *e.g.*, Jenner, Ehrlich, etc. (or the haemoglobin will be dissolved out), but not Romanowsky, Giemsa or other watery stains.

3. For other methods, and especially for the fixing of 'wet films,' *vide* ch. xxxiv.

TO STAIN FILMS

For the study of blood parasites some modification of the Romanowsky stain as developed by Ziemann, Leishman, Giemsa, etc., is by far the best, because by these stains the chromatin of nuclei is stained red while the protoplasm is stained blue and so a differentiation is got which is given by no other stain. The red nuclei, moreover, makes the detection of the parasite very easy.

ROMANOWSKY STAINS

The principle on which these stains are based is the following. A solution of methylene blue that has been acted upon by carbonate of soda or other reagents becomes partly converted into various derivatives, *e.g.*, methylene azure and methylene violet. These bodies also occur in old 'ripe' polychrome blues. These bodies are in solution. When they are acted upon by a solution of eosin there results a precipitate and this precipitated body (or bodies) possesses the property of staining nuclei an intense heliotrope red colour (chromatin stain).

The staining may be effected at the time of mixing the ripened blue and eosin solutions, in the nascent state, so to speak, as in method I; or the precipitate may be allowed to form, and subsequently be dissolved in a solvent, *e.g.*, methyl alcohol, as in the Leishman and Giemsa stains; and finally be precipitated out of solution by the addition of water at the time of staining.

1. *Romanowsky*.*—Two stock solutions are made,

* This method gives excellent results, and is not inferior to the Leishman or Giemsa stains. The stain is easily made and is cheap, and the blue, if made up, improves with age.

taking care to use the particular brand of stains specified.

Solution A.—Methylene blue (pure)* 1·0 part
Sodium carbonate* - 0·5 „
Ordinary water - 100·0 parts

Keep in the sun, or by the kitchen fire or in a hot incubator for two or three days until the solution has a distinct purple tinge. The solution is useless until this has developed. The solution improves with age and exposure to sunlight.

Solution B.—Eosin† - 1 part
Water - 1000 parts

This solution should be kept in a cupboard as it becomes gradually decolorised by sunlight.

For use take 5 c.c. of each stock solution and make each up to 100 c.c. with ordinary water.

To Stain.‡—Mix one part of blue and two of the eosin solution and pour on the slides. Stain ten minutes. Wash in a full stream of water. Blot and dry but do not heat. The platelets and nuclei should be a deep ruby red without any trace of blue.

Note I.—As it is the precipitate which results from the action of the ‘azure,’ etc., on the eosin, which is the active staining principle, this mixture must be used fresh, at least a few minutes only after making.

Note II.—While ten minutes or less is quite sufficiently long for staining with a good brand of stain, yet for certain purposes it may be necessary to stain for one or more hours.

* May be got in solid form from Burroughs & Wellcome.

† The eosin is water-soluble, of a yellow shade, or in other words pure eosin for blood work, e.g., the brand known as B.A. It may be got in tabloid form.

‡ Mix the solutions in the proportion of 1 : 1, 1 : 1·5, 1 : 2, etc., and ascertain which is the best proportion for each particular brand of stain, e.g., by staining a trypanosome, malaria parasite, etc.

Note III.—The longer a film is washed the more blue comes out of the red cells which eventually become pink, but at the same time the red chromatin stain of the nuclei of parasites, etc., is also partly dissolved. Blue may be extracted more readily by using dilute spirit. A film that has been washed too much or insufficiently stained can easily be restained (first dissolving off cedar wood oil with xylol).

2. *Leishman's Stain.*—Is the precipitate resulting from the action of a watery solution of *alkalised* methylene blue on a watery solution of eosin. The precipitate is filtered off, washed and dried. It is then dissolved in pure methyl alcohol.

(Leishman's stain in 'soloids' 0·015 grammes, methyl alcohol 10 c.c.; or the solution may be bought ready made, but its keeping properties are doubtful.)

To Stain.—(a) Without fixing, with a pipette, cover the film with the solution for one minute.

(b) Add about twice the quantity of water and mix carefully on the slide (with the pipette). Allow to stain five to ten minutes or for hours.

(c) Wash in water.

3. *Giemsa Stain.*—From the alkalised solution of methylene blue, Giemsa prepares in a pure condition some of the derivatives, *e.g.*, methylene azure (Azur I). An impure form of this, consisting of methylene azure and methylene blue in equal parts, he calls Azur II, and in fact it is this body that he uses in his stain, as the presence of methylene blue is necessary in order to stain the cell body. Further, he mixes with this an impure substance, Azur II-Eosin—which is the precipitate got by the action of eosin on alkalised methylene blue and which, therefore, consists of Azur I-Eosin, methylene blue-Eosin, with some methy-

lene violet. Giemsa's mixture (procured ready made) is then—

Azur II-Eosin	-	3	parts
Azur II	-	0.8	part
Glycerin	-	250.0	parts
Methyl alcohol	-	250.0	„

To Stain.—(1) Fix in alcohol. (2) Mix one drop of the solution with 1 c.c. of water. (3) Stain five to ten minutes, or several hours. (4) Wash in water.

Note.—To obtain malignant stippling (p. 33) it is recommended to add to 10 c.c. of solution one or two drops of 1 per *mille* potassium carbonate solution.

To obtain the most brilliant results with these stains is perfectly easy, and no one who has used them will, except for special reasons, use any others at present in use.

As stated above the simple Romanowsky stain is as good as any of the modifications.

To make fresh films.—For studying movement, delicacies of structure, for watching the process of ex-flagellation, phagocytosis, fertilisation, and other phenomena of the living parasite, it is necessary to be able to make good fresh films.

It is well to polish the slide and coverglass immediately before use with a clean handkerchief. When the exuding drop of blood reaches the size of a *small* pin's head, a coverglass is picked up rapidly with forceps, or *the edges* are grasped between finger and thumb, and allowed to touch the drop without 'dabbing' the skin, and then carefully dropped on to a slide. A gentle tap or two with a needle or forceps may aid in the film formation, but the pressure must not be great or the corpuscles will be found laked and invisible.

The requirements of a suitable wet film are.—That

there should be a central transparent area shewing no sign of a granular appearance, and even looking quite free from blood. If this appearance is present, the film is probably a good one. If the centre of the film appears reddish or granular, it is *useless* to examine it (for young parasites), as the corpuscles will be massed together and the parasite not seen. N.B.—Under the microscope a good film should shew clear, even, circular discs, lying side by side and not overlapping each other (Fig. 11).

Chapter III

THE DETECTION OF THE MALARIA PARASITE

N.B.—A stained specimen (Romanowsky) should always be used for the purpose of making a diagnosis as parasites are easily missed in fresh films.

To Examine the Stained Film.—After staining and drying, the film is ready for examination. *Canada-balsam and coverglass need not be applied.* A drop of cedar-wood oil is placed upon the film, and the oil immersion lowered into it.

Fix the right-hand end of the slide with a clip on the stage, and with the fine adjustment first focus *upwards* slightly, as the lens may be already too low. If the film does not come into view, focus downwards, keeping the unfixed end of the slide constantly moving very slightly to and fro with the left hand until the film comes into focus. If this precaution is taken a lens should never be jammed against a slide or driven through a coverglass.

Commence the examination at the *edge* of the film near the middle, and then proceed towards the points or tongues, for it is along the edge and especially in the tongues that parasites (if very scanty) will be found if present.

After the examination is completed, if it be desired to keep the film, the cedar oil is dissolved off by dropping a little xylol over the film and allowing this to drain off. and then to dry. After drying the

film can be put away and kept indefinitely. If not needed the slide is placed on one side with others, and eventually cleaned.*

THE DETECTION OF THE MALARIA PARASITE

We may first note that it is not necessary to examine the blood at any particular time, but it is very necessary that the patient should not have taken quinine previously. Even five grains of quinine may so diminish the number of parasites as to make detection a laborious task, and a negative result under these conditions is not conclusive.

The following forms of parasites may be seen :—

N.B.—Parasites free in the plasma are practically never seen.

1. *Ring Forms* (Fig. 6).—These may be quite small, one-sixth of a red cell in diameter, or much larger, one-third in diameter.

Rings are parasites of very distinct outline and structure. The part of the parasite that will first be noticed in a Romanowsky specimen will be the red nucleus, a clearly stained bright red dot (or dots). This is generally situated on the margin of the blue ring, which is equally distinct in outline, though often only a faint blue. The blue ring encloses an unstained 'vacuolic' area. These rings stand out so sharply that they appear to project from the corpuscles. The red dot generally forms the signet of the ring (signet forms), but also may occur in the centre of the vacuole. The red nucleus or dot is often also

* TO CLEAN DIRTY SLIDES

1. Rub with turpentine (benzine or xylol) to remove any adherent oil.
2. Wash with soap and water. 3. Rinse in water. 4. Dry and rub well with a clean cloth.

rod-shaped or angular. The rings may shew a very faint blue outline or a thicker portion on the side opposite to the nucleus.

Besides these young rings, we have irregular forms of considerable variety, *e.g.*, a mere faint bluish line stretching across the corpuscle, yet always shewing

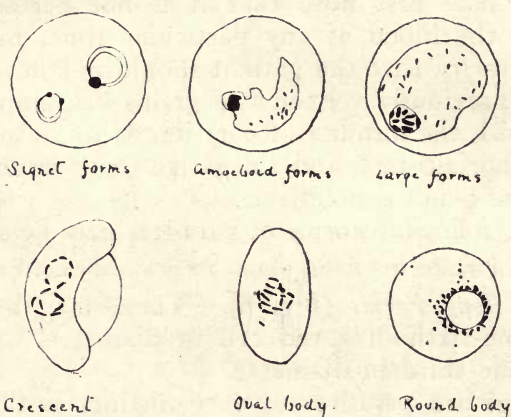


Fig. 6. Forms of the malaria parasite commonly met with in the blood:—The dark dots in the first line represent chromatin, the fine dots, pigment

somewhere a red nucleus; or, again, a mere streak along the margin of the red cell, with, however, a red nucleus in the blue protoplasm (*accolé* forms).

Finally, no small structure should be diagnosed as a parasite unless it is clearly made out that it has three distinct parts.

- (i) A red nucleus.
- (ii) A white vacuolic area within the ring (in the irregular forms this cannot be distinguished).
- (iii) Blue protoplasm or body.

2. *Large Intra-corpuseular Forms* (Fig. 6).—They appear as more or less extensive areas of blue protoplasm, with one or more distinct red areas. Pigment may be seen scattered over the parasite. These large forms are generally simple tertian or quartan parasites.

3. *Crescents and Spherical Bodies*.—These are most definite bodies, and readily recognized by the *coarse pigment granules* centrally situated. The presence of this pigment should absolutely preclude the possibility of mistaking distorted red cells, crescentic in shape, or a crescentic mass of platelets, for parasites. In neither of these is there a definite central pigment mass, nor should a foreign body be mistaken for a crescent. Moreover, crescents again have quite definite outlines, and shew a red-stained central portion and blue extremities.



Fig. 7. *Pigmented Large Mononuclear Leucocytes*

The same criteria apply to the spherical form of the crescent.

4. *Pigmented Leucocytes* (Fig. 7).—Large leucocytes with a large nucleus. Pigment (melanin) may occur scattered about the periphery of the cell or in little clumps, or even in very fine powdery grains. The pigment is brownish-black in colour. Skin pigment may be seen in epithelium scales or free in the specimen, but the definite position of the pigment in the protoplasm of the leucocyte characterizes melanin.

BODIES THAT MAY BE MISTAKEN FOR PARASITES

1. *Platelets*.—A single platelet lying on a red cell is often taken for a parasite. It is often quite round, but it is granular in appearance, and stains uniformly red or blotchy purple. There is no distinct separation into red nucleus, white vacuole and blue protoplasm.

Platelets lying *free* may show a great variety of shape, round, oval, sausage-shaped, in masses from the size of one-fifth to three or four times that of a red cell. They often, too, appear to have a clear outline, but the resemblance to a parasite is only superficial. There are no distinct areas, and, further, free parasites are *practically never* seen. Again, crescentic masses of platelets are often taken for crescents; but, again, there is no red chromatin and separate blue protoplasm, and no pigment.

2. *Stained Vacuoles*.—Artefacts of this kind generally occur in almost every cell in some portion of the field while quite absent in others. This is, of course, not the case with parasites. They are granular and much like a platelet, but lack the 'red, white and blue' of a parasite.

3. *Leucocytes*.—Are not uncommonly mistaken for large forms of parasites, *e.g.*, gametes, but if it is remembered that the greatest amount of chromatin in a gamete is always quite small, while, comparatively, the nucleus of a leucocyte is immense, this mistake cannot be made.

4. *Basophilia of Red Cell*.—Occasionally mistaken for a red cell shewing Schüffner's dots, containing a simple tertian parasite. Closer observation shews that the punctate cell contains no parasite.

5. *Normoblasts*.—The red cell contains a nucleus

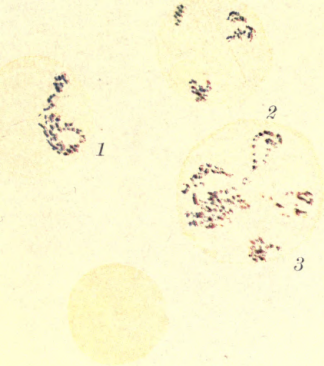


Fig. 1

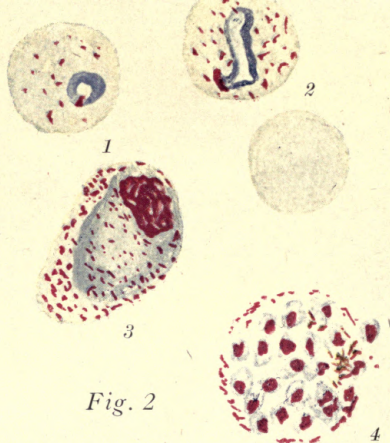


Fig. 2

BENIGN TERTIAN



Fig. 3

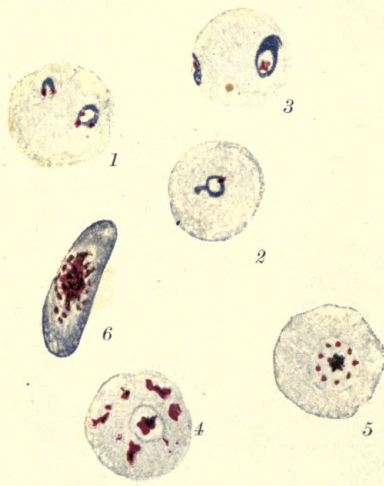


Fig. 4

MALIGNANT TERTIAN



Fig. 5

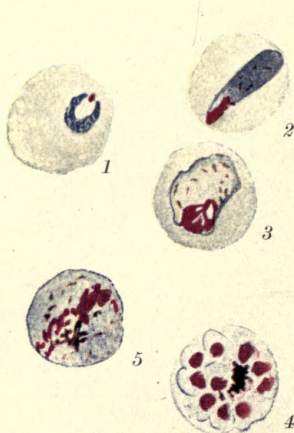


Fig. 6

QUARTAN

PLATE I


- Fig. 1.—Simple tertian parasite. Fresh preparation.
1 and 2. Young forms, pigmented, actively amoeboid.
3. Large form with 'boiling' pigment. Corpuscle much enlarged.

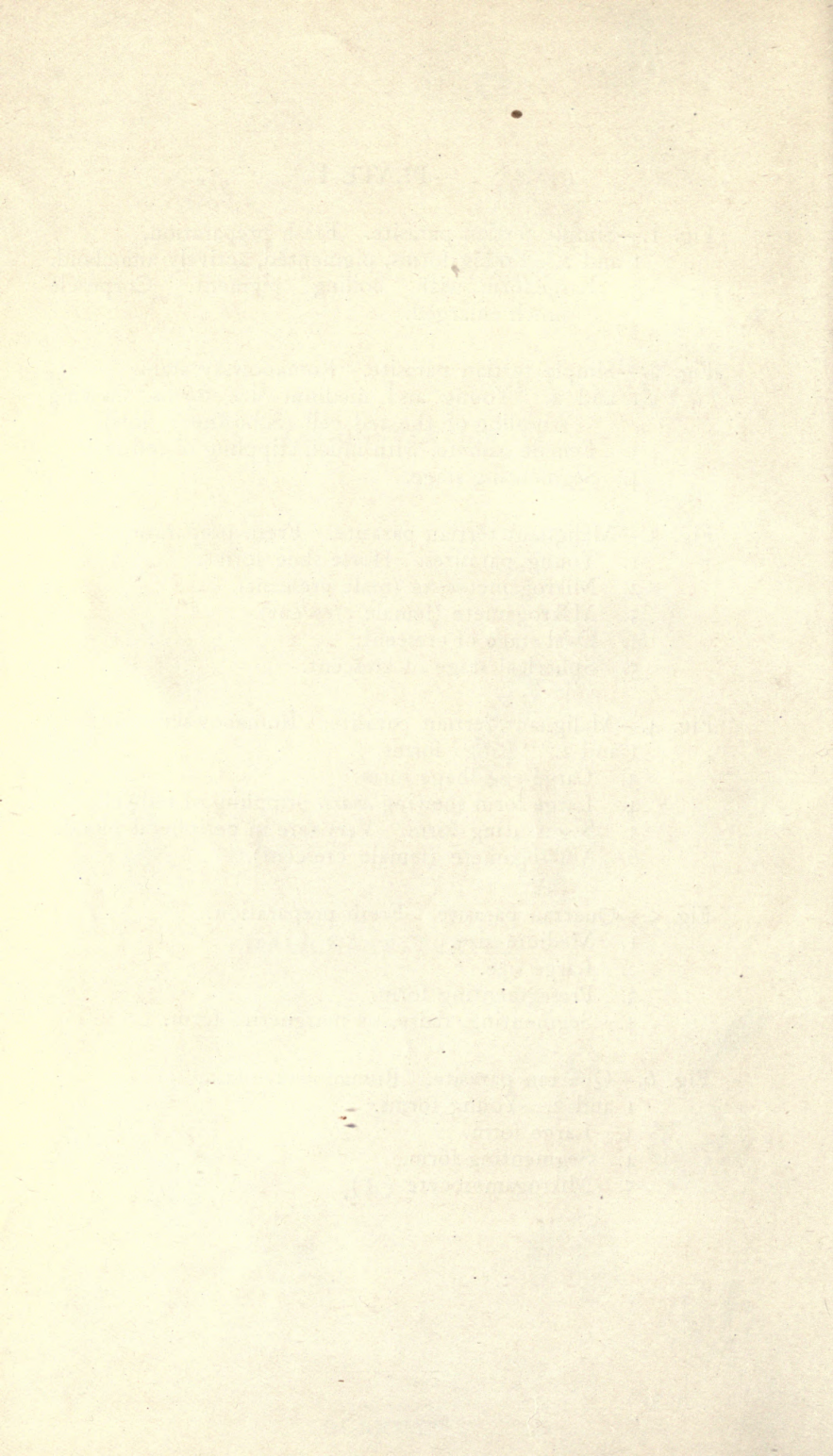
- Fig. 2.—Simple tertian parasite. Romanowsky stain.
1 and 2. Young and medium size forms, shewing stippling of the red cell (Schüffner's dots).
3. Female gamete, with much stippling of red cell.
4. Segmenting stage.

- Fig. 3.—Malignant tertian parasite. Fresh preparation.
1. Young parasites. Horse-shoe forms.
2. Mikrogametocyte (male crescent).
3. Makrogamete (female crescent).
4. Oval stage of crescent.
5. Spherical stage of crescent.

- Fig. 4.—Malignant tertian parasite. Romanowsky stain.
1 and 2. 'Ring' forms.
3. Large egg-shape form.
4. Large form shewing *coarse* stippling of red cell.
5. Segmenting form. Very rare in peripheral blood.
6. Makrogamete (female crescent).

- Fig. 5.—Quartan parasite. Fresh preparation.
1. Medium size.
2. Large size.
3. Presegmenting form.
4. Segmenting, daisy, or marguerite form.

- Fig. 6.—Quartan parasite. Romanowsky stain.
1 and 2. Young forms.
3. Large form.
4. Segmenting form.
5. Mikrogametocyte (σ).
- 



and even basophil punctation, but there is no blue protoplasm of a parasite.

6. *Skin Contaminations*.—Brownish yellow or even black pigment from the skin should not be confused with malarial pigment (melanin). Melanin practically never occurs free in the blood, but always in the protoplasm of leucocytes. Stained micrococci, yeasts, etc., are not uncommon, especially in the tropics.

7. *Squashed Leucocyte Nuclei*.—Frequently in malaria films (stained) large open meshworks of nuclear matter are seen with little or no surrounding protoplasm. These are degenerated or dropsical, or, according to others, mechanically damaged leucocytes, and often occur in great numbers.

8. *Deformed Red Cells*.—Further, we must point out an extraordinary appearance of the red cells in stained films. In anaemic (malarial) bloods we find red cells ten, thirty, or forty times the diameter of a normal cell, and these huge swollen structures shew at one side a crescentic area which is granular, and is the only remaining part of the red cell that can be recognized; the remainder is practically unstained. These gigantic structures may or may not be occupied by parasites. They are probably caused by the spreading of the film.

TO DETERMINE THE SPECIES OF PARASITE PRESENT

Three forms are recognized—simple tertian, malignant tertian, and quartan. The malignant tertian can, as we shall see, produce a quotidian temperature with only a single generation of parasites. Whether or no there is a true quotidian parasite, one or more, is extremely doubtful.

Malignant Tertian.—1. The rings of this species are the smallest, one-sixth to one-seventh the diameter of a red cell. Instead of being ring-like they may stretch across the cell (bacilliform), or be applied to the margin of the cell (Plate I). Pigment is generally absent, at least in the tropics. It must, however, be admitted that it is difficult to be certain of the species

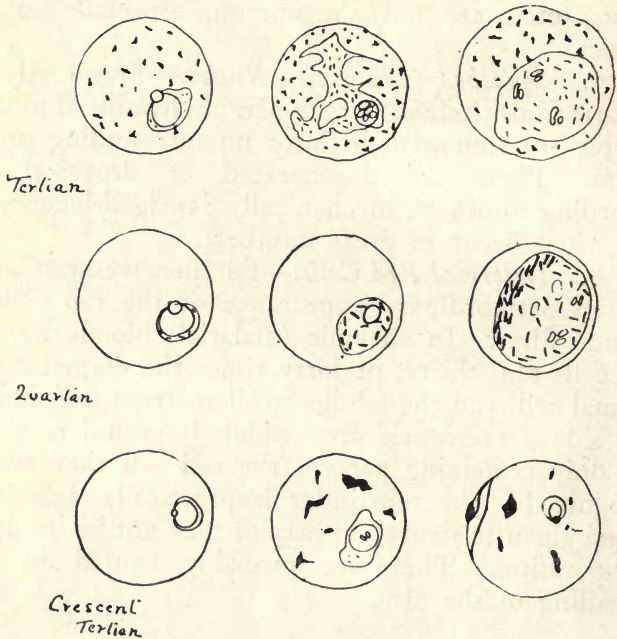


Fig. 7B. The three species of Malarial Parasites (stained)

of a single isolated ring. 2. The largest stage in the blood is characteristically egg-shaped, one-third to one-fourth the red cell. These forms occur (in regular charts) when the temperature is low. If, on the contrary, the temperature is still high, and large forms are found, they are probably simple tertian or quartan. 3. The infected red cell shews occasionally

(but it is difficult to get this staining effect) a characteristic coarse stippling quite different from that of the simple tertian. These consist of a few coarse dots or clefts around the parasite (Plate I, fig. 4).

The finding of crescents is, of course, diagnostic of malignant tertian, but the possibility of a double infection, *e.g.*, simple and malignant tertian, must be borne in mind.

Simple Tertian.—1. Characteristic is the fact that the infected red cell is distinctly larger than usual. 2. Characteristic also is the fact that the red cell is dotted all over with fine red granules (Schüffner's dots). Young forms as well as old produce this change in the red cell in a well-stained specimen, not overwashed. 3. The young rings are larger and more flimsy-looking than the previous. Pigment may be seen; it must be noted, however, that pigment is often obscured by Romanowsky stains. 4. The large forms are pigmented, irregular, flimsy-looking, and appear often as if consisting of two separate parts. This irregularity is characteristic (Plate I).

Quartan.—1. The red cell is unchanged. 2. The rings are compact, not irregular, and shew pigment early, but the diagnosis of the species of young rings is very difficult. 3. Larger forms are compact, and characterized by the peculiar dense nature of the chromatin, which is, relative to the size of the cell, plentiful. 4. Pigment often occurs as a dense streak along the margin. These parasites once seen are easily recognized again (Plate I).

We have so far described the forms generally encountered during a febrile attack and the means of making a diagnosis, but it is necessary to consider other forms, *e.g.*, the sporulating forms, and more especially the gametes.

In the presegmenting forms the pigment has not yet condensed into a single block, and the distribution of the chromatin masses is still irregular. In fresh preparations the typical 'daisy' forms can be clearly seen (Fig. 8).

GAMETES

Gametes are sexual parasites. They completely fill the cell, and are distinguished from adult asexual forms (Schizonts) by (1) their larger size, (2) by more abundant pigment, (3) by the fact that there is only one fairly large chromatin mass, whereas in an asexual form of nearly equal size the chromatin has already

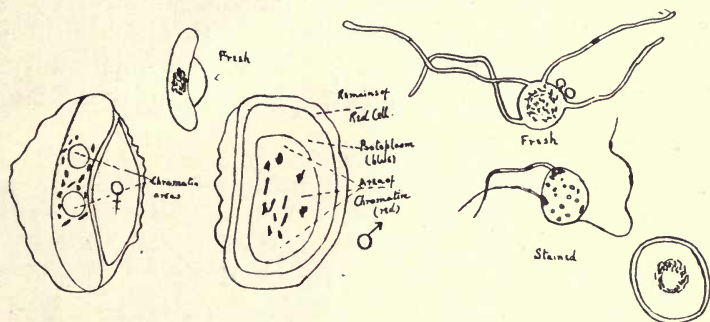


Fig. 9. *Malignant Tertian Gametes* (partly after Maurer).
Right-hand corner: Spherical body (fresh)

begun to divide into several portions (presegmenting stage). The different staining reactions of male and female are practically identical for all species. The ♂ being the fertilizing cell contains much nuclear matter staining red, while the ♀, being the nutritive cell, contains much protoplasmic matter staining deep blue.

1. *Malignant Tertian* (Fig. 9).—

MICROGAMETOCYTE (δ) MALE
CRESCENT

1. The chromatin of the nucleus occurs in an extensive loose network, occupying the greater portion of the parasite.
2. Comparatively little blue staining protoplasm.
3. The pigment is scattered.
4. The shape is somewhat kidney-shaped, shorter and broader than in the φ .

MACROGAMETE (φ) FEMALE
CRESCENT

1. The chromatin occurs in a compact mass more or less centrally placed.
2. Much more blue staining protoplasm.
3. The pigment is concentrated into a ring around the nucleus or heaped up into a mass.
4. Crescentic in shape, longer and narrower than the male.

N.B.—Gametes (crescents) may be but rarely found in the blood of Europeans in the tropics (West Africa).

2. *Simple Tertian*.—The female gamete (φ) is characterized by the possession of (1) much protoplasmic matter, staining *deep blue* with Romanowsky, (2) little compact chromatin laterally placed, and

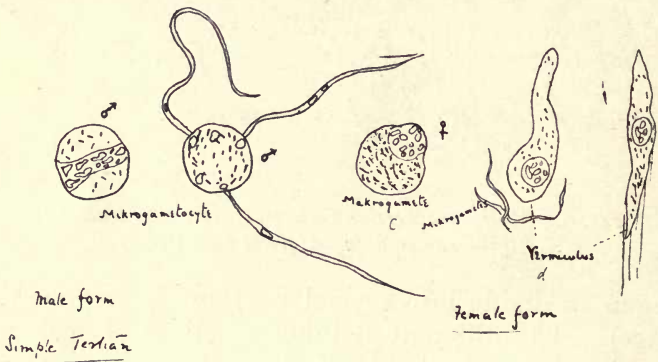


Fig. 10. Male and Female Simple Tertian Gametes
(after Schaudinn)

generally surrounded by a thin vacuolic area, (3) pigment black in colour, and irregularly scattered over the whole protoplasm (Fig. 10).

The male gamete (σ). (1) The chromatin is more voluminous than in the female, it is of a looser texture; that of the φ being compact is centrally placed or extends in a broad band across the cell. (2) It stains a characteristic greyish-green or greyish-red colour with Romanowsky, has little blue, so that the pigment is clearly seen, yellowish-brown in colour, while the female stains a deep blue, more deeply than the schizonts (*i.e.*, asexual forms) (Fig. 10).

3. *Quartan*.—Gametes are often very rare, presumably because the asexual fever stages may proceed with a clockwork regularity for months at a time. The σ has much voluminous chromatin, and the protoplasm stains light blue. The φ has a small amount of compact chromatin and stains deep blue.

APPEARANCES IN FRESH SPECIMENS

N.B.—It is impossible to detect young parasites unless the films are thin and uniformly spread with the cells lying side by side (Fig. 11). If the cells overlap or are deformed in any way it is waste of time searching for young rings. In a properly spread specimen the red cells appear as uniform straw-coloured discs with a central paler area.

1. *Young Parasites*.—The most characteristic features which distinguish parasites from other appearances in the red cell are (*a*) the characteristic opaque white look, like that of a white cloud, the definite contour, and the fact that the central portion of the parasite is of the same colour as the red cell which is in fact seen through its substance. The ring has often a thickening at one point, giving the 'signet ring' appearance. (*b*) On watching such a ring from time to time it is seen to have definitely altered its shape. (*c*) Pigment, perhaps only a grain or two may be seen, but in malignant

tertian rings, especially in the tropics, pigment may be quite absent. (*d*) It is extremely easy even with the keenest attention to overlook young parasites, hence for diagnosis a stained specimen is the best.

2. *Large Parasites*.—It is clear that the cell is occupied by a hyaline body, a parasite; and the pigment often in motion, in some cases extremely fine, is obvious on careful examination.

3. *Crescents and Spherical Bodies* (Fig. 9).—The former are, as in the stained specimen, characterized by their shape. They are distinct, fat, plump-looking bodies, unmistakable when once seen. They always have, besides, a central clump of distinct pigment. Stretching across between each end of the crescent is seen the curved edge of the red cell. The spherical bodies also possess this definite, easily seen pigment mass.

4. *Pigmented Leucocytes*.—A body should not be diagnosed as a pigmented leucocyte unless it is first clearly made out that the body is a leucocyte, *i.e.*, possesses a distinct nucleus and cell substance. The pigment may consist of one or more black spicules or larger clumps evidently lying in the protoplasm.

An epithelial cell is a flat-looking cell with a relatively small nucleus. Adherent skin pigment has not the fine acicular or granular character of melanin, and does not lie in the protoplasm (Fig. 7).

BODIES IN FRESH FILMS THAT MAY BE MISTAKEN FOR PARASITES

1. *Vacuoles*.—Vacuoles and cracks have not the opaque look of parasites. The former cannot be focussed sharply, they 'open out.' Cracks have often

a peculiar reddish tinge or refraction about them (Fig. 11).

2. *Crenations*.—Focus alternately as dark and bright points. They have no clear ring outline (Fig. 11).

3. *Platelets*.—Appear as somewhat indistinct granular masses and do not possess the opaque white look of parasites. The 'definiteness' of a parasite is one of its chief characters.

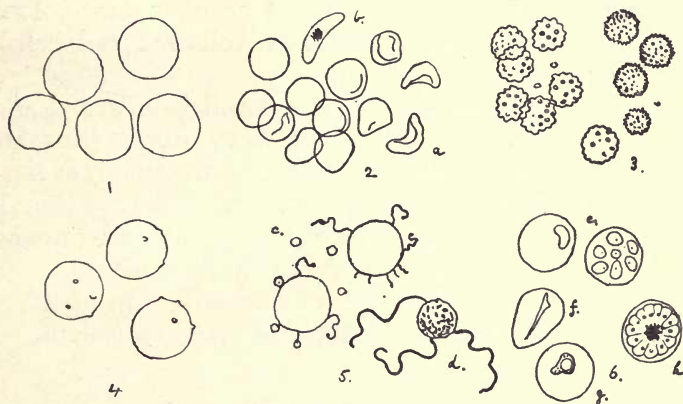


Fig. 11. 1. Thin portion of film. 2. Thicker portion : (a) a distorted corpuscle with no pigment ; (b) a crescent with pigment. 3. Crenated corpuscles. 4. Commencing crenation. 5. (c) Red cells with processes ; (d) a male gamete with flagella and pigment. 6. (e) vacuoles ; (f) a crack ; (g) a young parasite with very fine pigment ; (h) a segmenting parasite with central pigment mass

4. *Eye Spots*.—Bi-convex in shape with a central dot representing the pupil. They are seen in normal blood, but have often been described as new varieties of parasites.

5. *Red Cells with Long Wavy Processes*.—These are seen especially in anaemic bloods after the fresh

film has been under examination for some time. The processes occasionally break off and float about. Shorter and more granular processes emitted by the red cell are even commoner (Fig. 11).

DISTINCTION OF SPECIES IN FRESH FILMS

Malignant Tertian.—The young rings are the smallest seen. The red cells in which they occur have sometimes, especially perhaps in severe infections, a slightly crenated appearance and peculiar dark colour (globuli rossi ottonati, old-brass coloured red cells). Pigment is rare.

Simple Tertian.—1. The red cell is enlarged and pale. 2. The young rings are more flimsy (but are often extremely difficult to see), and the medium sizes may shew several pseudopodia. 3. Their motion is greater. 4. The pigment is very fine, reddish brown; in the larger forms it is more easily recognizable.

Quartan.—1. Red cell unchanged. 2. Motility slight. 3. Pigment compact and dark chocolate.

FLAGELLATION

Select a case of malignant tertian* infection in which parasites have been found. On examining the patient about a week later, crescents (gametes) will be found in the blood. In about twenty minutes, or in hot weather in England in five minutes or less, many of these will be seen to become spherical and to get free of the corpuscle in which they were situated. Two varieties may be distinguished—the male, in which the pigment is distributed over the whole of the parasite, and the female, in which the pigment is

* Flagellation may of course be studied also in simple tertian and quartan.

concentrated into a ring or figure of 8. Observe that attached to these spherical bodies small ring-like bodies occur, one to two in number, about as big as a pin's head. These are the so-called 'polar' bodies. They occur in the male and female, and, as seen in stained specimens, consist of little circular masses of chromatin (?). These changes occur in the tropics very rapidly, so that the examination must be commenced as rapidly as possible.

On watching these spheres, the pigment in some will be seen to be in active motion—this probably indicates the internal changes preparatory to extrusion of flagella. Suddenly one of these spheres is perceived to be oscillating violently, and in a moment three or four or more pale, long processes are emitted (Fig. 9). The red cells all around are put in motion by their violence, and it may be only after a time, when the activity has grown less, that the flagella are actually seen. Nodosities will be observed in the flagella, and occasionally a speck of pigment at their extreme end. The flagella after a time break off, but they have only once, by MacCallum, been seen penetrating the female gamete.

TO STAIN FLAGELLATED BODIES

When flagellation is observed the coverglass is forcibly 'smeared' off; slide and coverglass are then fixed, and stained with Romanowsky.

Beautiful preparations are easily got by this method (Fig. 9).

THE SUBSIDIARY SIGNS OF MALARIA

When patients have taken quinine it is not uncommonly impossible to find parasites in the peri-

pheral blood. Apart from the actual presence of the parasites, one may still derive evidence of malarial infection from—

1. The presence of pigmented leucocytes.
2. An alteration in the proportion of the leucocytes.

Pigmented Leucocytes.—Pigmented leucocytes, even in severe malaria are often very few, and often require for their detection prolonged search in large films. In other cases, however, they are abundant. The presence of very few is quite compatible with a severe malarial infection. For instance, in two cases seen by us, only very few were found in the peripheral blood, but in the spleen, post-mortem, enormous numbers occurred. To detect them it is necessary to make large and good films by the method already described. By following the margins and termination of the film, the majority of leucocytes in the film will have passed beneath the eye, and pigment, if present, is readily seen.

In the vast majority of cases the pigment will be found in the large mononuclear forms, and only very rarely indeed in the polymorphonuclear forms. As a rule, a pigmented large mononuclear (Fig. 7) is crowded with granules of pigment, the presence of only a few grains, or a single granular clump, is exceptional. The appearance of the clearly defined yellowish-brown or black pigment granules in the clear protoplasm is so characteristic that no doubt ought to exist. It should be remembered, however, that in dirty films specks of dirt may be over a leucocyte, and so resemble pigment. In this case similar specks will be found lying free. The occurrence of malarial pigment free in the blood has never been seen by us.

Leucocytic Variation.—Often in cases where pigmented leucocytes are difficult to find there is a very obvious increase in the percentage of the *large mononuclear leucocytes*. This change, which is usually very pronounced in the apyretic periods of an attack of malaria, is, however, most frequently absent during pyretic periods. If, during a period of low temperature, this change is not found, there is a strong presumption that the case is not malarial. If the blood be taken at the height of the fever, a negative result does not exclude malaria, and a further examination should be undertaken, if possible, during an apyretic period. In some cases the change can be detected even during the pyretic periods, but in these it is always more marked in the apyretic. In some cases, during the course of the fever, no such change occurs, but appears immediately the temperature subsides, and diminishes as convalescence proceeds. Perhaps the cases where this test is of the greatest value are those where the patient has already been treated with quinine, and one can scarcely hope, even if the disease be malaria, to find parasites in the blood.

From the results obtained by blood counts of a considerable number of Europeans living in the tropics, we found that an increase beyond fifteen per cent. of the large mononuclear forms is proof of an actual or recent malarial infection, whereas with a value of twenty per cent. it is almost always possible, by long search, to find an occasional parasite or pigmented leucocyte. A value of over twenty per cent. probably implies actual infection at the time of observation.

Chapter IV

THE PARASITE IN THE TISSUES

To Make Smear Preparations.—Place a minute portion of the tissue (*e.g.* brain, spleen) on a slide, and with the end of another slide spread it out as evenly and thinly as possible. Dry, fix, and stain in the same way as a blood film. Parasites, if present, are in this way much more easily and clearly seen than in sections. Spleen pulp, bone marrow, kidney, liver, etc., give beautiful results, and in the same way any secretion or fluid can be examined.

Fixation of Tissues.—N.B.—Use small pieces.

1. Cut the tissues with a sharp knife or razor into pieces *not thicker* than one-eighth of an inch (3 mm.)

2. Place some cotton wool at the bottom of a bottle or specimen tube. Pour in the fixative and add the pieces of tissue. The amount of fixative to be used should be about fifty times the volume of the tissue.

3. Pieces of intestine (*e.g.*, in cases of *malaria cholERICA*) may be laid on pieces of paper and then put in the fixative. The tissue adheres to the paper and retains its shape. Proceed similarly for any thin tissue or thin slice of an organ which it is required to embed and get sections of rapidly.

4. The results got with alcohol or formalin are not nearly as good as with other fixatives which require some trouble in the making. (*Vide* ch. xxxiv.)

Time Required for Fixation.—This varies according to the size of tissues and fixative, but the necessary time can always be determined by cutting through one of the pieces. If the tissue is opaque throughout, it is a sign that fixation is complete. The tissue should then be removed and washed. (*Vide* ch. xxxiv.)

Embedding Apparatus.—A slab of metal (copper), $12 \times 3 \times \frac{1}{4}$ inches. Heat this at one end, and place the vessel containing the paraffin at a point on the slab where the paraffin is just kept melted. This is

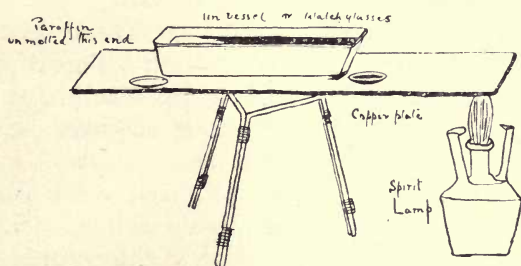


Fig. 12. Simple Embedding Apparatus

the temperature for embedding. This simple device serves all the purposes of an elaborate paraffin oven (Fig. 12).

Microtome.—The Cambridge rocking microtome (£5), or the Minot (£12) are the most convenient, but sections can be quite well cut with cheaper instruments, e.g., the Jung (£1 10s.).

Razors.—These may be hollow-ground on one side, or on both, to a varying depth. For general use a moderately hollow-ground razor is used. Examine the edge under a low power to see if any notches exist if so they must be ground out on a hone. A Belgian stone, as long as possible, should be used and kept free from grit during use. The stone should be soft,

capable of being scratched with a pin, and as a lubricant water or filtered kerosene oil may be used. If the razor is hollow-ground on one side only, it should be honed only on this side. After honing, the razor should be stropped. On one side of the strop a *minimum* amount of razor paste should be rubbed in and the leather side should be kept scrupulously clean and dry.

Examined under the microscope the edge should now present a clear, sharp line. It may be tested on a thin hair, which it should easily cut.

Clearing.—1. Dehydrate the tissue by placing in quite water-free absolute alcohol. This is prepared in the following way: Heat copper sulphate crystals in a basin until all trace of blue is gone. Cool and add to the alcohol in the bottle.

2. Pour some ordinary* cedar-wood oil into a specimen tube. (Or the tissue when dehydrated may be placed in a tube of xylol or chloroform or other clearing agent until quite transparent.) Pour on top of this some water-free absolute alcohol.

3. Place the tissue in; it floats at the junction layer of the alcohol and the oil.

4. Allow to remain until it sinks into the oil. It is then ready for transferring to paraffin.

Choice of Paraffin—For the most delicate work, a paraffin with a low melting point (45°) is necessary, but in the tropics a much harder paraffin is necessary, *e.g.*, one melting about 60°C . Superheated paraffin (Count Spee's), melting about 56° , gives excellent ribbons, when the room temperature is low enough to allow of its use.

To Embed Tissues.—1. The tissue, now transparent,

* Not immersion oil.

is taken out of the cedar-wood oil and the excess wiped off in order to prevent the oil softening the paraffin.

2. Place in a dish of melted paraffin for half an hour, more or less according to the size of the tissue. (If on removing and allowing the block to cool a cut made with a razor through the tissue looks quite uniform, it is a sign that the paraffin has penetrated properly).

3. Arrange two brass blocks (L pieces) on an ordinary slide so as to form a trough of the required dimensions. Melt some paraffin in a dish and fill up the trough to the top. Now transfer the tissue with a warmed forceps to the trough and arrange as required. (As soon as the surface becomes opaque and semi-solid, the position of the object, if necessary, may be marked by scratching with a needle on the paraffin.)

4. When the surface is solid, but not before, cool under a tap of water.

5. Trim the block by cutting away successive slices. Take care that the sides are parallel. (Block trimmers are very useful where continuous series of sections are to be cut.)

6. Put some paraffin on the block holder, warm in flame and now press on the paraffin block firmly until it adheres. Melt bits of paraffin around the base with a hot knife to give it further support.

Treatment of Sections.—1. Place a trace (pin-head size) of egg albumen and glycerine on a slide and rub it carefully over with the finger (free from grease).

2. Just cover the slide with water from a pipette.

3. Lift a section (or ribbon if required) from the microtome with a strip of note paper. Avoid touching the knife. Now with the needle carefully draw the ribbon on to the water and repeat the operation until

the slide is covered with a series of ribbons (if desired) one beneath the other. Warm the sections very carefully over a small flame until the paraffin is stretched.

4. Drain off the excess of water and then blot off the remainder. Dry the sections in an ordinary incubator 38.5° (about); or by a fire, protected from dust; or, for rough purposes, over a flame without melting the paraffin wax.

5. Allow xylol to trickle over the section till the paraffin is dissolved out, or place in a pot of xylol.

6. Wash off the xylol in alcohol and then bring (through descending strengths of alcohol) into water. (*Vide* ch. xxxiv.)

STAINING

Haematein.—Stain five to twenty minutes according to the activity of the stain as seen by the depth of colour of the sections. If over-stained they may be decolorized in one per cent. alum solution. Wash in water. Counterstain with eosin (p. 408) for one minute.

Methylene Blue.—Stain with one-fourth per cent. methylene blue for one hour. Wash in water and alcohol and counterstain with eosin.

Romanowsky.—It is difficult to get good results with sections as indeed it is with thick blood films. Formalin should not be used to fix. The sections should be as thin as possible. Fülleborn recommends the following method.

(1) Giemsa stain, 10 drops, H_2O 20 c.c. Stain with two or three lots for several hours.

(2) Wash off excess and then drop on acetic acid, 1 in 1000, till violet. Wash in water.

(3) Stain with the ordinary diluted Romanowsky (for one hour).

(4) Place in seventy per cent. alcohol and gradually increase strength till absolute alcohol containing some eosin is added.

(5) Xylol. Balsam.

To Mount Sections.—Pass through successive alcohols, oil of cloves (or xylol), to Canada balsam. In hot moist climates, the cold produced by the evaporation of the alcohol causes dew to be deposited upon the slide. When the xylol or oil of cloves is added, this produces a troublesome milkiness and may spoil the section. To avoid this, all excess should be rapidly wiped up after the use of alcohol, and the oil of cloves added as quickly as possible.

POST-MORTEM CHANGES IN MALARIA

(MARCHIAFAVA AND BIGNAMI)

Brain :—

1. Punctiform haemorrhages of the meninges.
2. Punctiform haemorrhages of the white substance of the brain.
3. The brain capillaries may have nearly every red cell infected. Sporulating forms are especially common.
4. The capillary endothelium may show fatty degeneration, together with pigmentation, and sometimes parasites.
5. Similar appearances are also found in the vessel of the pia mater.

Lungs :—

1. Large pigmented mononuclears in the capillaries, but especially in the veins; in the lungs especially, phagocytosis is proceeding.

2. There is a terminal infection with the diplococcus pneumoniae.

Spleen.—The trabeculae of the pulp are distended by infected red cells, and pigmented large mononuclears are abundant. The malpighian follicles, on the contrary, are non-pigmented.

Liver.—Endothelium of capillaries is swollen and pigmented. Pigment is also found in Kupfer's cells. The liver cells contain only haemosiderin, not melanin. Pigmentation is most intense around the central veins.

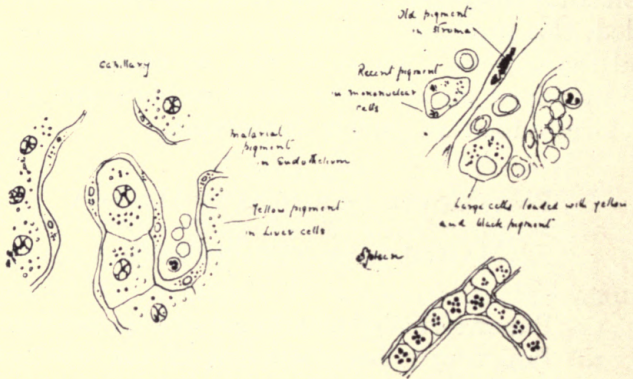


Fig. 13. Shewing deposition of Pigment in Liver (left), Spleen (right), and Sporulating Parasites in Brain Capillary (bottom)

Kidneys.—Pigmentation is much less marked. Changes may occur in the epithelium of the tubules, independent of the presence of parasites.

Bone Marrow.—Parasites and melanin, free, and in large mononuclear leucocytes, and macrophages are found. Crescents may be found here when absent of scanty elsewhere, as in the spleen and brain; it is consequently supposed that they principally develop here.

In cases of malaria of long standing the yellow marrow becomes red.

Stomach and Intestines.—In malaria with choleraic or haemorrhagic symptoms, parasites may abound in the capillaries of the villi.

CHRONIC MALARIA

Spleen.—As is well known, the spleen may in these cases fill the whole abdomen. Dilatation of the various lacunae occurs with a thickening of the splenic reticulum. The pigment tends to become deposited eventually in the connective tissue surrounding the follicles. The splenic septa become thickened.

Liver.—The pigment is found mainly in the *periphery* of the lobules, and pigment in the form of blocks in the perivascular connective tissue.

The capillaries are much dilated, and the epithelium contains blocks of pigment. Atrophy of the liver cells and their nuclei occurs.

Bone Marrow.—The marrow of the long bones is usually red, due to a large development of haemoblastic tissue. Normoblasts are common.

Pigment disappears rapidly from the bone marrow.

LITERATURE

Marchiafava and Bignami. *Twentieth Century Practice of Medicine*. Malaria. Vol. XIX. S. Low, Marston and Co. This comprehensive and learned treatise is incomparably the best in the English language, dealing with all aspects of malaria and also black-water fever.

Chapter V

THE MALARIAL PARASITE

LIFE HISTORY

The Protozoa are divided into several classes, one of which is the Sporozoa.

This class comprises several orders, the most closely allied of which are the Gregarinida (*e.g.*, *Monocystis agilis* in the seminal vesicles of the earth worm), the Coccidiidea (*e.g.*, *Coccidium oviforme* in the rabbit's liver) and the Haemosporidia (which include the malarial parasites of man, birds, etc.). There is a close relationship between the Coccidiidea and the Haemosporidia (malaria parasites), the developmental cycles of the two being almost identical. The developmental cycle in the blood (the febrile cycle) of the malaria parasites was first demonstrated by Golgi, the further cycle in the mosquito by Ross. The cycle of Golgi is the asexual cycle, producing auto-infection of the patient ; the cycle of Ross is the sexual cycle, producing a new infection in a healthy subject.

The sexual cycle, it has been thought, commences in the blood when the conditions are unfavourable for the continuance of the asexual cycle, and, in fact, has been taken as a sign that the patient has already developed immunity against the fever-producing young parasites (spores). Thus it is well known that in malignant tertian the sexual forms, gametes or crescents, first appear about a week to ten days after

the first febrile attack. If this view be true, then it follows that the gametes develop from forms already present in the system, viz., the asexual forms, and possibly the divergence into sexual forms takes place from the youngest form of the parasite, *i.e.*, the spore. But it is possible that the divergence takes place at a stage previous to the youngest form of parasite, *i.e.*, at a stage immediately succeeding the entry of sporozoits into the blood, so that we have from the first indifferent and sexual forms present, involving, indeed, the existence of three kinds of sporozoits. Sexual development has been supposed to proceed mainly in the internal organs, *e.g.*, bone marrow ; but it is being gradually recognized that young forms of gametes are also found in the circulation. Let us suppose that we are now dealing with fully developed gametes in the blood. We shall proceed to describe the further changes undergone in the mosquito. The male cell is, as we have seen, called the microgametocyte ; the female cell, the macrogamete. These we can distinguish in the blood. Further flagellation can be observed, *i.e.*, the protrusion of so-called 'flagella,' *i.e.*, microgametes or spermatozoa. These 'flagella' break off and fertilize the female cell, the macrogamete, a process which has been seen in *Halteridium* of birds, but only once in man.

This fertilized female cell or egg is known as a *Zygote*. At a slightly later stage it is called the *Vermiculus* or *Ookinete* (Figs. 10 and 14). Both these terms are suitable ones, for the first describes the fact that the zygote becomes worm-like in shape, and the second, that the zygote moves. The *Vermiculus* stage can be seen on the slide in the case of *Halteridium*, but in the case of malaria parasites, only by taking the blood from the stomach of the mosquito after a suitable

lapse of time. The Vermiculus now finds its way through the epithelium of the stomach, and then lies in the external muscular layers as a spherical or ovoid body. A kind of capsule is formed around it by these

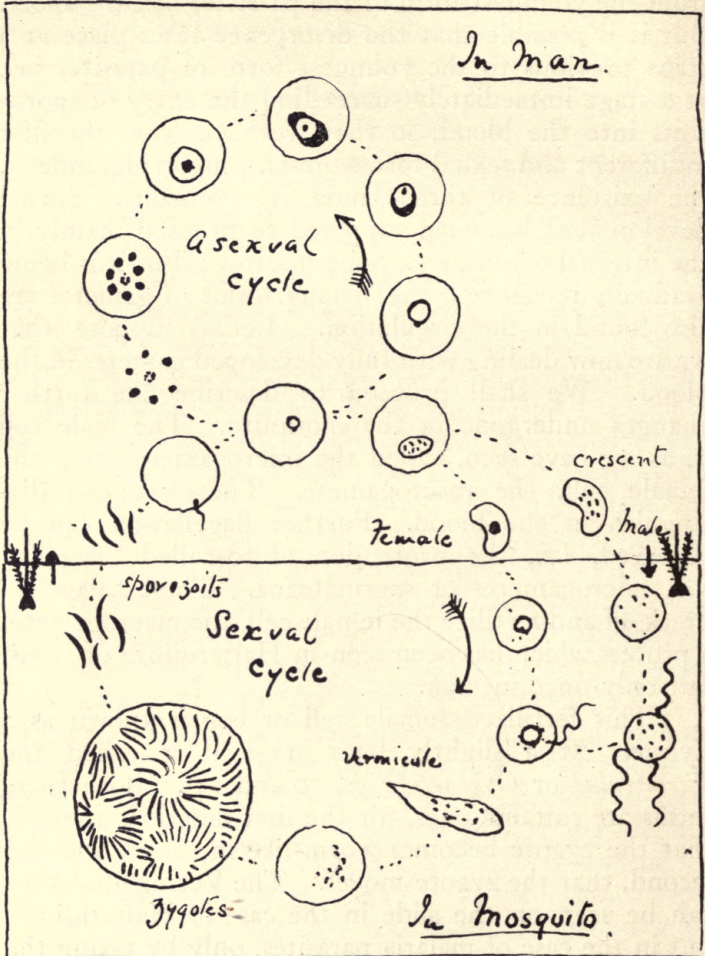


Fig. 14. Life Cycle of the Malaria Parasite in Man and the Mosquito.

tissues, and so at this stage it is also called the encysted zygote or *Oöcyst*. Growth proceeds, and signs of division into several masses appear in the protoplasm. These masses are termed *Sporoblasts*. Then we reach the stage of large oocyst (with sporoblasts), and by this time the masses of the sporoblast have undergone division into a number of fine curved thread-like bodies, the *Sporozoits*, so that eventually the large cyst is almost entirely filled with sporozoits. The capsule of the cyst eventually ruptures, and the sporozoits pass from the tissues of the stomach to the thorax, being found at first amidst the muscles, but eventually all collected in the salivary glands. From here they are injected into the blood by the mosquito, and they then attach themselves to and penetrate the red cells (as has been actually observed under the microscope by Schaudinn), producing a new infection.

We may briefly summarize these various steps :—

1. Microgametocyte, and macrogamete in blood.
2. Development of microgametes = flagellation, on the slide and in nature in stomach of an Anopheline.
3. Fertilization of the macrogamete producing a zygote or copula, on the slide and in nature in the mosquito.
4. Vermiculus or ookinet. Only in mosquito stomach.
5. Oocyst. In stomach wall.
6. Medium or large oocyst* with sporoblasts.
7. Sporozoits. (i) In the large cysts, (2) in salivary glands.

The sexual cycle is known also as sporogony or amphigony, while the asexual cycle is known as schizogony or monogony. These two cycles and their

* Not uncommonly stages 4-6 are also called zygote.

relation to one another are shown in the figure (Fig. 14).

Further, there is a certain amount of evidence to shew that a gamete (♀) in the blood can undergo a kind of retrogressive development, and give rise by parthenogenesis to young parasites (*i.e.*, schizonts). If this is so, it would explain the supposed function of old attributed to crescents (gametes) of producing relapses.

There is no evidence at present that the parasite passes into the ovum, giving rise to hereditary transmission.

Chapter VI

MOSQUITOES

Mosquitoes belong to the order of Diptera, or true flies, which are characterised by :—

1. A single pair of membranous wings.
2. Suctorial mouth.
3. Complete metamorphosis (egg, larva, nymph).

They differ from all other flies in that they have *scales* on the wings and body.

In all mosquitoes, except the genera *Corethra* and *Mochlonyx*, there is a long piercing proboscis, which is characteristic of the *Culicidae*.

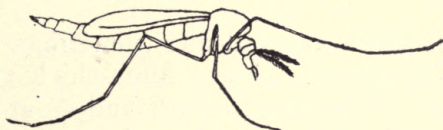


Fig. 15. *Chironomus*

Flies which may be mistaken for mosquitoes are :—

1. *Chironomidae* (Midges), e.g. *Chironomus*.—Costal vein does not extend beyond the tip of the wing. Antennae of male densely feathered. Legs long and slender (*vide* ch. xxxiii). They do not possess the characteristic proboscis of mosquitoes. The veins of their wings are more complex, and are *quite devoid of scales* (Fig. 15).

2. *Tipulidae* (Daddy long-legs).—Some small *Tipulidae* often possess a considerable superficial resemblance to mosquitoes, as, for example, the winter gnat (*Trichocera*), the wings of which are spotted. When at rest their bodies lie parallel with the surface, and upon it. They have no distinct proboscis (Fig. 16).

3. *Cecidomyiidae*, or gall midges.—These have a simple wing venation, and there are no forked cells. In most species the wings and bodies are hairy, not scaled.

4. *Rhyphidae*.—Wings have a discal cell (below the anterior cross vein). They may have spotted wings.

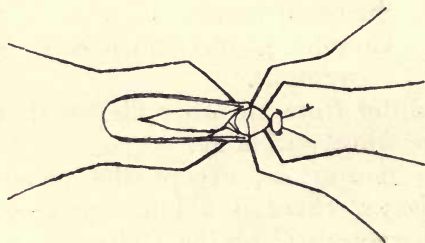


Fig. 16. *Trichocera*

5. *Simulidae*, or sand-flies (sometimes also called midges).—These are minute flies which suck blood voraciously. They have a short and stout proboscis. The salivary glands are very large in proportion to the size of the fly, and the bite is as severe as that of a mosquito. The males are harmless (Fig. 17).

The larvae of the *Simulidae* are aquatic, cylindrical in shape, and live on the stems of water plants. The imago hatches beneath the water.

6. *Psychodidae* (or owl midges), e.g., *Phlebotomus*.—Small fluffy-looking flies which suck blood readily. They are most readily detected after feeding, when the abdomen is swollen with blood. They have very hairy

wings and body, and a short powerful proboscis (Fig. 17). The larvae are aquatic but can also exist in air.



Fig. 17. Sand Fly (left).



Owl Midge (right)

LIFE HISTORY OF THE MOSQUITO

In common with all other insects shewing complete metamorphosis, the mosquito passes through four stages :—

- The egg.
- The larva.
- The nymph.
- The imago.

The Imago.—The imago is the well-known winged insect. The emergence of the imago may be seen on the surface of almost any collection of foul water. For some considerable time after hatching (twenty-four hours) the insects refuse to feed.

In the imago there are marked differences between the male and the female insect.

The Male.—In the male the antennae are markedly plumose. The palps also are long and hairy. The effect is to make the 'head' of the male mosquito very conspicuous (Fig. 18).

The male mosquito does not feed upon blood, and the proboscis is only used to suck vegetable juices.

The Female.—In the female the antennae are inconspicuous and have only short lateral hairs. The palps are also less conspicuous than in the male (Fig. 18).

The female feeds upon blood, and is frequently seen with the stomach distended with blood, more or less digested.

The female is also seen with the abdomen more or less swollen, with the greatly enlarged ovaries, which give a whiteish and opaque colour to the mosquito, and often make the insect much more conspicuous in its flight than it otherwise would be.

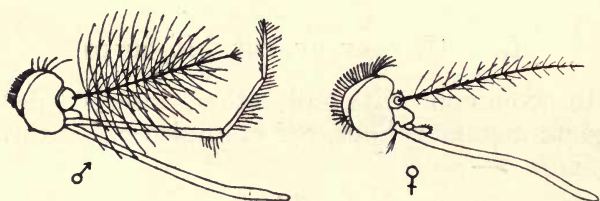


Fig. 18. Heads of Male (♂) and Female (♀) *Culex*

The *Culicidae* or mosquitoes are divided into several sub-families, e.g., *Anophelina*, *Culicina*, etc.

The sub-family, *Anophelina*, is in many ways the most distinct of these groups. Not only are the adult insects highly characteristic in appearance, but the ovum and larva are quite unlike those of any other sub-family.

The points which serve to distinguish the *Anophelinae* from other groups of mosquitoes are:—

1. The character upon which the sub-family is founded, viz., the relative length of the palps and proboscis. In both the sub-families, *Culicina* and *Anophelina*, the palps in the *male* are long plumose structures, as long or longer than the proboscis. In

the *female Culicinae*, however, the palps are quite short and insignificant structures, whereas in the *female Anophelinae* these are scaled and as long as the proboscis. An examination of the female proboscis will at once determine whether an insect belongs to the sub-family *Anophelina* or other sub-family.

2. The wings in nearly all species of *Anophelines* are 'spotted,' but this is only a popular and not a scientific criterion. By the use of the low power of the microscope or an ordinary lens, these spots are seen to be due to the presence of areas of dark scales upon the wing veins, elsewhere covered with light scales.

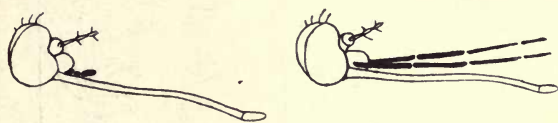


Fig. 19. Shewing distinction between palpi of Female *Anopheline* (right) and a *Culicine* (left)

There are, however, a few members of the *Anophelinae* which have not spotted wings (e.g., *A. bifurcatus*, and the Indian *A. immaculatus*). Also there are other mosquitoes than *Anophelines* which have spots, e.g., *C. mimeticus* (costal spots), also mosquitoes of the genera *Theobaldia* and *Lutzia*. Nevertheless, as a general rough rule, mosquitoes with *spotted wings* are *Anophelines*.

3. The angle which the proboscis makes with the rest of the body is very different in *Anophelines* from that of other mosquitoes. In *Culex*, *Taeniorhynchus*, or *Stegomyia*, the proboscis forms a distinct angle with the line of the body (*Taeniorhynchus*, forty-five degrees). In the case of *Anophelines*, the proboscis continues on in the line of the body (*P. stephensi*, fifteen degrees). The result is to give to an *Anopheline*

mosquito a peculiar and very characteristic awl-like appearance (Fig. 20).

4. The attitude adopted by *Anophelines* is, as a rule, characteristic. When an *Anopheline* rests upon a wall, its body projects so as to form a distinct angle with it. In some cases the angle assumed is almost a right-angle. In the case of almost all other mosquitoes the body is held either parallel with the wall, or what is more frequent, the tail approaches the wall, giving the insect a 'hunchbacked' appearance. This differ-

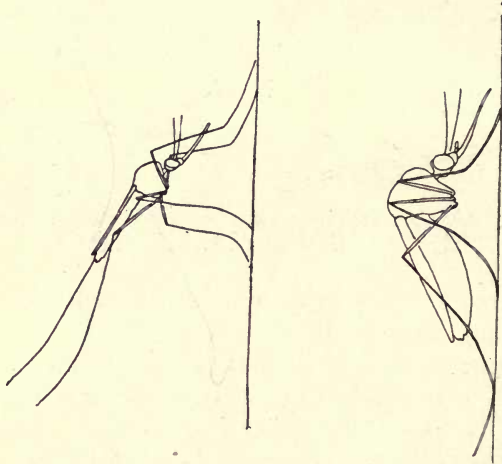


Fig. 20. Shewing distinction between resting attitude of an *Anopheline* (left) and *Taeniorhynchus* (right)

ence is readily seen by any careful observer, and is a practical and useful distinction. A characteristic of an *Anopheline* is that it rests by preference on the first two pairs of legs only, and keeps the last pair stretched out *stiff* and *straight*, or they slowly oscillate to and fro. Many mosquitoes wave the hind legs, notably *Stegomyia*, but they are held with the tarsi curved upwards.

The exact attitude adopted depends upon the species and the situation, whether a vertical or horizontal surface on which the *Anopheline* is resting. One very common species (*M. culicifacies*) at least, when sitting on a wall, looks exactly like a small brown *Culex*, since it holds its body parallel with the wall as a *Culex* does.

Culex.—Mosquitoes of the genus *Culex* are many of them brown mosquitoes of sober hue, e.g., the common house *Culex*, *C. fatigans*, which is uniformly brown without markings. The genus, however, contains a very large number of species. In *Culex* mosquitoes the attitude when resting is 'hunchback.'

Stegomyia.—The genus *Stegomyia* is of the greatest interest and importance, since it is the one which is concerned in the transmission of yellow fever (*Stegomyia calopus* v. *fasciata*).

These mosquitoes are generally black and white, with banded legs and abdomen, and spots on the thorax. They are found in houses, and are most troublesome mosquitoes from their habit of feeding in the day, and their great alertness and persistence. *Stegomyia* are also very common in woods and forests.

CAPTURE OF MOSQUITOES AND FLIES

I. Place a lamp upon a sheet of white paper, and note the insects which are attracted by the light. Note insects belonging to the orders Lepidoptera (moths), Hemiptera (aphides, green flies, etc.), Heteroptera (plant bugs), Neuroptera (caddis flies, stone flies, white ants). Pick out any mosquito-like flies. They will probably belong to the Chironomidae. Note that true mosquitoes are not seen around the lamp.

2. Examine, with a light, some wall which has been only dimly illuminated by the lamp, *i.e.*, some wall at the distance of several yards, and note true mosquitoes resting upon this. Capture several of these by placing a tumbler over them, and kill them by puffing in a little tobacco smoke.

The specimens caught will probably be specimens of *Culex*. If near a swamp or jungly place there may be *Taeniorhynchus*, *Mansonia*, and possibly *Anophelines*. Observe the hunchback attitude in the case of most of the mosquitoes caught. If an *Anopheline* should by chance be caught, note the striking difference in the general appearance, the attitude, and the spots on the wings.

3. Observe in stuffy, furnished rooms, offices, etc., the presence of mosquitoes feeding actively during the day. Capture some of these. They will probably belong to the genus *Stegomyia*. Note their extreme alertness. Observe that they are black with white bands. Note the habit of waving the hind legs, and that the tarsi of these are kept curved. Ascertain whether the males feed upon blood.

4. Examine stables, huts, outhouses, bridges, drains, etc., in the early morning.

LITERATURE

The Cambridge Natural History. 'Insects, Part II.' A most useful book for an introductory knowledge of a variety of winged life in the tropics and elsewhere.

Chapter VII

THE OVUM

Ova are minute bodies one mm. or less in length. When first laid they are white in colour but rapidly become brown or black. They occur on the surface of water, and if submerged do not hatch out. Mosquito eggs may be laid by the edge of water, or on floating objects, or upon the water. In the last case, they have some device to ensure that they shall float, and not sink and be destroyed. In the case of *Anopheles* and some species of *Stegomyia* (Fig. 22), each ovum lies separately upon the water, and has air cells which keep it afloat. In the case of *Culex* and *Taeniorhynchus*, hundreds of eggs are cemented together to form rafts, each egg lying perpendicularly, with its larger end pointing downwards. In *Culex*, the egg-rafts are broad and roughly oval in shape (Fig. 21). In *Taeniorhynchus*, the egg-raft is extraordinarily elongated, resembling, in shape, a racing skiff (Fig. 23).

CULICINAE

Culex.—Examine the surface of some semi-putrid water for egg-rafts of *Culex*. Egg-rafts can almost always be found on the surface of water containing macerating leaves, fruit, etc. They are bodies of a blackish-brown colour, and are readily wafted about by the wind.

1. Note that the raft is boat-shaped, measuring one-fifth to one-third inch in length, and consists of two hundred to four hundred eggs.

2. Note that the separate ova are smooth elongated bodies, about 0.7 to 0.8 mm. in length. Note that there are no floats or other markings as in the case of *Anopheline* ova.

3. Note that one end of the egg is thicker and blunter than the other, and that to the thicker end is attached a clear transparent globular body (the micropilar apparatus). Note that this body is readily detached, often leaving a spike-like process projecting from the thicker end of the ovum.

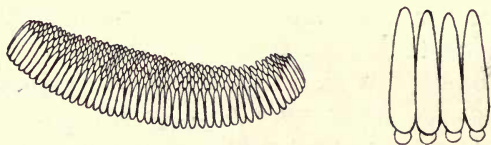


Fig. 21. Egg Raft and Eggs of *Culex*

4. Make as many observations as possible upon the egg-rafts, *e.g.*, time necessary for hatching of larvae, amount of desiccation they will withstand.

Taylor, in Havana, has made many observations on eggs. He gives the following:—*C. pipiens*, raft 200-400 eggs; egg 0.9 by 0.16 mm. *C. nigrifolius*, raft 200-300 eggs; egg 0.6 by 0.14 mm. *U. lowii*, raft 50-75 eggs.

5. The egg stage in *C. jamaicensis* lasts twelve hours; in *C. sollicitans*, twelve hours.

Stegomyia.—Confine some gravid females of *Stegomyia* mosquitoes.

1. Note that in *S. calopus*, *v. fasciata* the eggs are laid singly, about fifty in number, and much resemble, at first sight, the ova of *Anophelines*. Note that in others the eggs are laid in rafts (*S. notoscripta*).

2. Note that they are irregularly oval, thicker at one end than the other, and have a corrugated surface in which are entangled numerous minute air bubbles.

3. Examine the surface of water left exposed for several days in a tumbler, etc. Note, if *Stegomyia* mosquitoes have ovi-posed, the presence of eggs occurring singly or in parallel groups. Note that the ova are larger than those of *Anophelines*, and that they hatch into *Culicine*-like larvae.

4. The egg stage in *S. calopus* lasts twelve to twenty-four hours.



Fig. 22. Eggs of *Stegomyia*

5. The eggs of *Culicidae* have but little resistance to desiccation, but those of *S. calopus* will hatch after being kept 'dry' for three months.

Taeniorhynchus.—Examine natural waters, especially small pools with a dense growth of alga, swamp pools, irrigated land, etc., for the egg-rafts.

1. Observe the extreme length and narrowness of the rafts. Note also how small a portion of the raft is submerged.

2. Observe that the ova are arranged as in *Culex* rafts with the thicker end downwards, and that they are smooth and have a micropilar apparatus.

3. Endeavour to obtain the ova of known species of *Taeniorhynchus*, by confining gravid females. Note the shape of the rafts.

Mansonia.—Observe that the eggs have a curious snout-like projection, and that they are laid singly.

Psorophora.—The eggs are large, two mm. long. They occur in patterns like those of *Anophelines*. The eggs are covered with minute prickly scales.

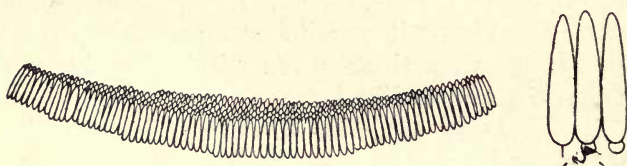


Fig. 23. Egg Raft and Eggs of *Taeniorhynchus*

ANOPHELINEAE

Anophelinae.—The ova of *Anophelinae* are difficult to detect in nature, but may be seen by the aid of a lens on the margins of small pools, where larvae abound. They are about 0·7 to 1·0 mm. long.

1. Confine some female *Anophelines* as described on p. 190. Endeavour to choose those in which the ovaries are nearly mature (p. 113). Fifty to one hundred and fifty eggs are laid. Remove the piece of paper upon which the ova have been deposited and place this upon a slide. Examine with a low power in strong daylight, and the mirror turned off.

2. Observe the remarkable resemblance of the ova to little boats, and the presence of the two beautiful oval air cells placed upon either side, acting as floats. (These are absent only in two species as yet described, viz., *M. turkhudi* and *M. azriki*). Observe also the presence of a white frill or a mere ribbed rim around what would be the gunwale of the boat (Fig. 62).

3. Observe that one end of the ovum is always stouter than the other. The stout end contains the head of the embryo, and is the end from which the young larva escapes. Note also that when *Anopheline* eggs are seen at the side of vessels drawn up by capillarity the thick end is at the bottom. Examine the surface of the water remaining in the hollow stopper or receptacle, and observe that the ova of *Anophelines* are laid singly without any cement substance, and float singly or touching one another on the water.

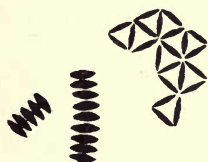


Fig. 24. Patterns formed by Eggs of *Anophelines*

4. Observe star-shaped patterns formed by some species, or the arrangement in parallel groups assumed by the ova of others (Fig. 24). Note that this arrangement is dependent on physical causes (shape of the egg, etc.), and not on the fact that the eggs are laid in such positions. This is readily done by stirring up a number of *Anopheline* ova on water, and noting how they tend to form groups in triangles and star shapes (p. 186).

5. Ascertain that *Anopheline* ova, when first laid, are white, but rapidly darken and become black. Observe that *Anopheline* ova are very often laid in heaped-up masses, which eventually become dispersed by waves, etc. Observe that the eggs then form patterns.

6. Place some half-dried mud in a flat dish, and put this inside a piece of mosquito netting in which

some *Anophelines* with ripe ovaries are placed. Observe that ova are laid upon the mud.

7. Preserve the mud for forty-eight hours, preventing it from becoming completely dry.

At the end of forty-eight hours or more, remove a few ova to a dry slide, and place under a low power. Allow a drop of water to flow on to the ova. Observe the escape, within a minute or so, of the young larvae, and the fact that a cap-like piece of the egg-shell is pushed off.

8. Observe that *Anophelines* kept in a dry test tube will occasionally lay their eggs on the side of the tube.

9. Note the time when the eggs were laid and the time at which the larvae emerge. This depends greatly on the temperature. It may take two or three days. *Ce. argyrotarsis*, one-and-a-half days (Taylor).

10. Remove *Anopheline* ova on paper and allow them to dry, and note that after two, or three days (86°-96° F.) at the most, they will not hatch when carefully placed in water.

Chapter VIII

THE LARVA AND NYMPH

THE LARVA

The larvae of mosquitoes, more especially of *Culex*, are well-known objects. They can be seen by holding up to the light almost any specimen of water in the tropics that has been left undisturbed for some days, but especially water which contains macerating leaves.

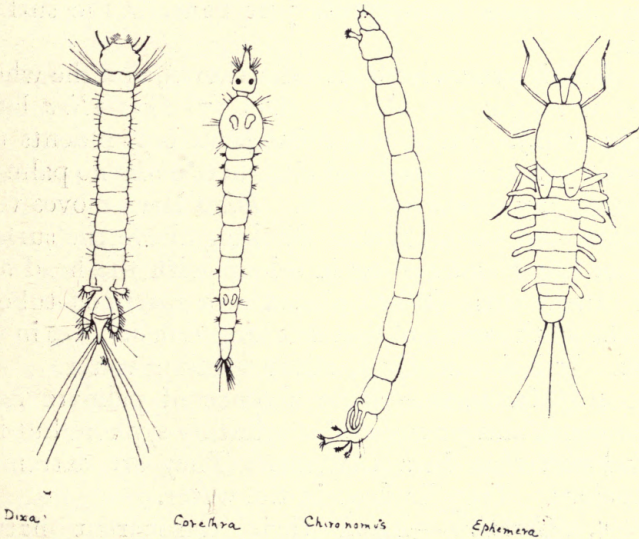


Fig. 25. Larvae that may be mistaken for Mosquito Larvae

Larvae which may be mistaken for those of mosquitoes are :

1. *Chironomidae*.—The larva of *Chironomus* is a red worm-like creature (blood worms). On the prothorax it has a pair of feet armed with hooks (Fig. 25).

2. *Ephemeridae* (May-flies).—The larvae of certain small *Ephemeridae* may, at first glance, be mistaken for mosquito larvae. There is no real resemblance, and the triradiate tail of the *Ephemera* larva and the tracheal gills distinguish it (Fig. 25).

3. *Dixidae*.—The larva of *Dixa* rather closely resembles the larva of *Anophelines*, though not other mosquito larvae (Fig. 25). Ventrally it has pseudo-pods on the fourth and fifth segments.

In its movement along the surface of the water the larva of *Dixa* resembles *Anopheline* larvae, and this larva also rests horizontally just beneath the surface film.

In *Dixa* there is no globular thorax, and the whole larva is longer and thinner than an *Anopheline* larva (eight mm.). Moreover, *Dixa* larva only indents the surface film at the head and tail, there being no palmate hairs on any of the segments. *Dixa* larva moves very rapidly, and has a habit of climbing above the surface of the water and resting in a loop with the head and tail downwards. When placed in a specimen tube it climbs up the side and becomes lodged in crevices in the cork. It is frequently found in running water.

4. *Mochlonyx*.—Note absence of palmate hairs on dorsum and presence of respiratory syphon, but less developed than in a *Culicine*. They are extremely voracious. They lie deep in the water.

5. *Corethra*.—From their transparent nature known as 'Phantom-larvae.' They have a very small

head, the front part of which is only half the width of the posterior portion. On the last segment there is a swimming fan. There is no respiratory syphon. They lie horizontally rather deep in the water and are extremely voracious (Fig. 25). Add some *Corethra* larvae to a glass of water containing *Culicine* larvae and watch the result !

THE LARVAE OF ANOPHELINES

To collect the larvae.—Necessary apparatus :—

1. An ordinary spoon.
2. A white enamel tin or large cup, or an ordinary bath tin.
3. Bottles, specimen tubes, paper, pencils, etc.

1. *By inspection.*—Inspect closely the surface of any small puddles that have been in existence some time. Examine especially small rock puddles, small shallow pools in 'nullahs' and river-beds, in the dry season.

Examine especially the edges where larvae are fond of resting, with the head facing the open water and the tail touching the bank. Note also how larvae tend to cling to floating twigs, etc. If no larvae are seen, stir up vigorously the bottom of the pool with the spoon. This will dislodge larvae from the edges, etc.

Examine the surface of the pool again and observe the larvae now plainly visible against the muddy water. Wait a few minutes for the appearance of the larger larvae, which remain below longer than the younger forms. Examine carefully for nymphae, which easily escape detection.

Dip out the larvae and nymphae with the spoon as they appear. The thinner the edge of the spoon the

less disturbance is caused, and the more readily are larvae removed.

2. *By Dipping*.—Choose any water with grassy or weedy edges, *e.g.*, the edges of rivers, streams, ditches, lake margins, swamps, etc.

With the least possible disturbance, dip out water from the most sheltered positions, and as close to the vegetation as possible, bringing up water and weeds in the can. Allow the specimen of water to remain a few seconds, and remove any larvae or nymphae as they appear on the top with the spoon.

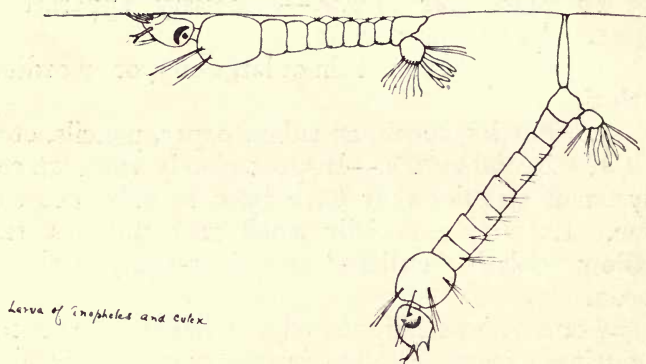


Fig. 26. Larvae of an *Anopheline* (left) and *Culex* (right)

Anopheline larvae should not be mistaken for any other mosquito larvae.

1. When undisturbed they lie flat along the top of the water, and on every segment certain hairs (palmate hairs) actually indent the surface film. Observe that when viewed in certain lights from one side these indentations can be plainly seen. The appearance may even be as though the dorsum of the larvae projected from the water. This, however, is not the case.

This appearance is diagnostic of the *Anopheline* larva. One species (*M. turkbudi*) does not, however, rest in this position, but after rising to the surface in a horizontal position slowly sinks until the tail only touches the surface.

Anopheline larvae, which are about to turn into nymphae, also sometimes tend to sink, so that the head is directed obliquely downwards (often seen in *M. rossi*).

One species at least of *Culex* (*C. concolor*) adopts a nearly horizontal attitude. The line of indentations of the surface film mentioned above, is not however seen.

2. When disturbed, *Anopheline* larvae dart into the water, or what is very characteristic, if not greatly disturbed, they pass by a series of wriggling jerks along the surface of the water.

When moving up towards the surface, an *Anopheline* moves in a much more irregular and jerky manner than a *Gulicine* larva.

3. *Anopheline* larvae, when full grown, possess very small heads in proportion to the size of the larvae (about eight mm. in length). In most of the *Gulicinae* the head is very large, with very prominent and large antennae.

4. *Anopheline* larvae have no syphon, the tracheae opening into a pit on the dorsum of the eighth abdominal segment.

Procure a considerable number of *Anopheline* larvae, and ascertain the following points:—

1. *The Moulting of Anophelines*.—Note that as *Anopheline* larvae grow in size they cast their skins. Remove a cast skin by floating it upon a slide. Note the perfect nature of the 'skin,' and how all the chitinous structures are represented, even air tubes.

Observe how beautifully certain hairs resembling fan-palm leaves are shewn (palmate hairs) (Fig. 65).

2. *The Method of Feeding of Anophelines*.—Observe with a lens the action of the feeding brushes and the currents they produce on the surface of the water. Note the rotation of the head so that, whilst feeding, the ventral surface of the head is uppermost.

3. *The Food of Larvae*.—Tear a larva to pieces with a needle and remove a small portion of the dark central mass of food material filling the straight alimentary canal. Place in a drop of clean water and crush under a coverglass. Note what organisms form the chief bulk of the food. Note the presence of sand grains, unicellular plants and animals, short lengths of alga, diatoms, bacilli, etc.

Determine the common foods of several species of *Anophelines*.

4. *Desiccation of Larvae*.—Celli and Casagrandi have found that *Anopheline* larvae can only resist desiccation at 20° C. for two days, at 35° C. for one day, and 40° C. for two minutes only. Larvae of *Anophelines* stranded on moist mud will live as long as four days, but in the tropics as soon as the mud loses its glistening surface they die.

5. *Cannibalism of Larvae*.—Add some large *Culex* larvae to a small bottle containing some small larvae or *Anopheline* larvae. The *Anopheline* larvae or small *Culex* larvae will be devoured by the large forms. *Mucidus* sp., *C. concolor*, and *Psorophora* sp., are especially cannibalistic.

6. Observe the occurrence in nature of *Culicine* and *Anopheline* larvae, also what *Culicine* larvae are found living together.

7. *The Enemies of Larvae*.—Add small fish, water-beetles (*Dytiscidae*, *Hydrophilidae*), and their larvae,

Libellula larvae, *Corysca*, *Nepa*, tadpoles, and other water animals, respectively, to a series of bottles containing equal numbers of larvae. Note the rate at which they are devoured, if at all. The carnivorous forms *Nepa*, *Corysca*, *Libellula* rapidly devour larvae. *Hydrophilidae* beetles, tadpoles, etc., do not destroy larvae. Observe that some species of fish are much more active devourers of larvae than others.

N.B.—Wherever possible introduce *small* fish into tanks, ponds, etc.

Note that weeds often protect larvae from being consumed by small fish.

8. Make experiments with different chemical and other bodies, and note the absence or presence of culicidal power.

(a) Note that chemical bodies in solution kill only with difficulty, as a rule; *e.g.*, corrosive sublimate. Ammonia, however (1 in 4,000 of water), will kill mature larvae according to Waddell.

(b) Note that oils rapidly kill larvae by blocking the air tubes. Treat larvae by pouring a little olive oil upon the water. Stain with osmic acid and note globules of oil within the air tubes.

9. Add some paraffin oil to a small *Anopheline* pool, observe the presence next morning of dead female mosquitoes that have come to lay their eggs. Observe the effect of paraffin on different kinds of natural water, and the great efficacy in some cases and futility in others.

10. Observe that pools covered with *Lemna* (duckweed) are very frequently, if not always, free from larvae. The action of the *Lemna* is said to be mechanical.

N.B.—Wherever possible grow duckweed in ponds, tanks, etc.

CULICINE LARVAE

The larvae of the *Culicinae* are superficially much alike. There are, however, marked differences in some features on closer examination. These differences are to be found mainly in the syphon tube, the antennae, the mouth parts, and mental plate, and to a less extent in other structures.

Note differences in naked eye appearance ; note the long worm-like *Stegomyia* larva and its wriggling mode of progression ; note the transparent and spiny appearance of some larvae (e.g., *Melanoconion* and *Taeniorhynchus*) ; note that some larvae adopt a nearly horizontal attitude (*C. concolor* and *Mucidus scataphagoides*) ; others a vertical attitude (*Stegomyia*) ; whilst the majority form a small angle with the vertical. Examine larvae under a low objective. Note the penultimate segment (eighth) which carries the syphon tube and a patch of scales known as the 'comb.'

Note especially the following :—

- (i) The syphon tube.
- (ii) The antennae.
- (iii) The mouth parts.
- (iv) The anal papillae.

The Syphon Tube.—This is formed of a single cylindrical piece of chitin, and contains the origin of the two main tracheae of the body. Note the small flap-like pieces of chitin forming a closing apparatus at the extreme tip. Measure the length and greatest breadth of the syphon tube ; note that in different species, and especially in different genera, the syphon tube varies greatly in its measurement. By dividing the length by the breadth a figure may be obtained which is useful in classification and may be termed the *syphonic index* number.

*Note two rows of spines, 'the pecten,' on the posterior aspect of the syphon tube, starting from the base and extending a variable distance up the syphon tube; note that they differ much in number and shape, etc., in different species. In some species (*e.g.*, in cannibal larvae) a large fan of hairs projects posteriorly in the median line from the syphon tube.

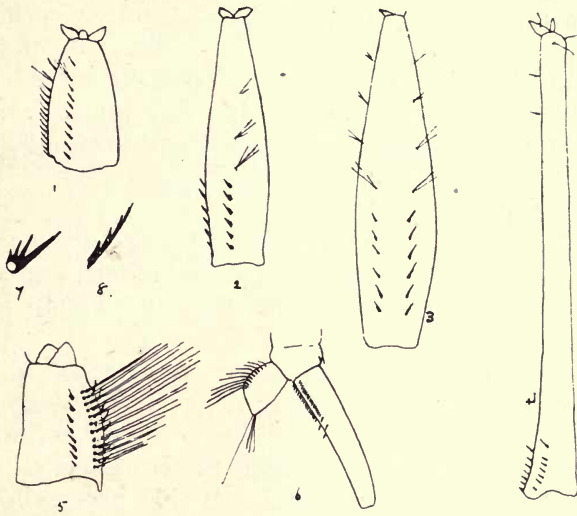


Fig. 27. Respiratory Syphons of Larvae.

- (1) *Stegomyia* sp. ; (2) *Culex* sp. ; (3) *Culex* with Large Syphon Tube ;
 (4) *Taeniorhynchus* sp. ; (5) Cannibal Larva (? *C. concolor*) ;
 (6) Enormous Syphon Tube (one quarter scale of others,
 genus undetermined) ; (7) Spine on Tube seen on
 the flat ; (8) Spine seen sideways

teriorly in the median line from the syphon tube. In certain species the syphon tube is of enormous size, and may attain to one-third the length of the larva. On the eighth segment at the base of the syphon is a group of scales, the 'comb.' It differs much in

different species and is highly specialised in the *Anophelinae*.

The Antennae.—The size and position of the antennal tuft or tufts is of systematic importance. Note the different position of these tufts in different genera of *Culicines*, e.g., *Taeniorhynchus* (*tenax*) and *Culex* (*fatigans*) (Fig. 28). In other cases there is considerable variety in position in different species of the same genus, e.g., *Culex*. Note the peculiar antennae of the cannibal larvae *C. concolor* and *Mucidus* spp., *Scatophagoides* spp. In *Stegomyia* (*calopus*) the antenna is small and spineless and possess a single hair about the middle (Fig. 28).

The mouth parts.—

(a) *The Clypeus*.—The front portion of the head projecting between the antennae is smooth and semi-circular in shape. The most anterior portion is the clypeus.

(b) *Feeding Brushes*.—These are attached on each side to the under surface of the clypeus. They resemble shaving-brushes and are employed in collecting the minute food particles on which the larva feeds.

In the fully grown larva a snout-like process bearing a tuft of hairs projects forward between the brushes.

(c) *Mandibles*.—Are stout chitinous structures one on either side and dorsal to the maxillae and maxillary palps. Each mandible bears stout hairs, for combing the shaving brushes, strong teeth meeting those on its fellow for masticating, and fine hairs which project into the mouth cavity.

(d) *Maxillae*.—They form a great part of the floor of the mouth. The maxilla is covered with series of hairs which serve the purpose of combing the feeding brushes.

(e) *Maxillary Palps*.—Articulating with the

maxillae lie on their outer edge and somewhat dorsally. At their tip they have spines and a membranous lamella. The palps serve to close in the mouth cavity.

(f) *Labial Plate*.—Or under lip of Meinert, is a conical toothed piece in the middle inferior line.

This latter (and probably also the mandibular teeth and maxillary hairs and palps) differs much in different species (Fig. 28). It is generally of specific but sometimes may be of generic importance.

The Anal Papillae.—Note the tracheae ramifying in these, the papillae being possibly gill-like in function. In *Megarhinus*, *Toxorhynchites*, *Mucidus*, *Psorophora*, *Lutzia*, *C. concolor*, and *C. tigripes* they are quite rudimentary.

Culex.—(1) Examine in a glass dish and observe the hanging attitude of the larva and how the angle varies in different species, e.g., in *C. concolor* it is nearly horizontal.

(2) Note with the microscope the absence of palmate hairs.

(3) Note the position and extent of the antennal tuft, and shape of the antenna. The differences are not so striking as in the case of the syphon.

(4) By the great differences in 'syphonic index' it is often possible to distinguish the different species.

Important differences occur also in the scales of the pecten and comb. It is possible to subdivide the genus into groups in which these characters are more or less alike.

Stegomyia.—The larva of *Stegomyia* is rather longer than that of *Culex*. When disturbed it exhibits a rather lashing movement like that of certain small aquatic worms. When at rest at the surface, the attitude of the body is almost vertical. The larva, however, spends a good deal of its time browsing at the

bottom of the water, and then lies for the most part horizontally.

The head is small in proportion to the rest of the body, and the thorax is less conspicuously marked off from the abdomen than in *Culex*.

The antennae resemble those of *Anophele* larvae, more than those of *Culex*. The large branched hair of *Culex* is represented by a short inconspicuous simple hair (or as many as three) projecting from the side of the antennae (Fig. 28).

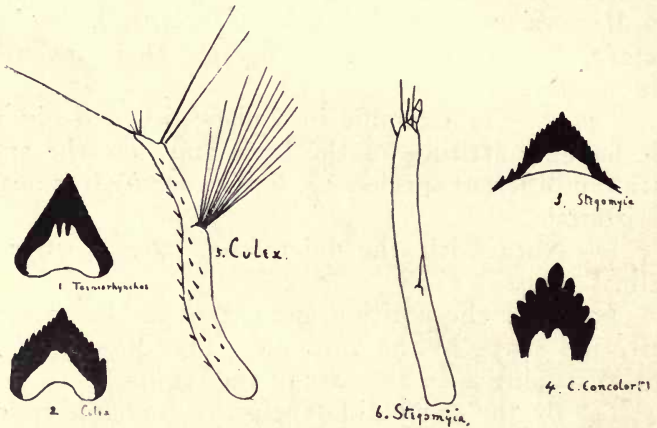


Fig. 28. Antennae and Mental Plates of Larvae

The syphon tube is dark in colour, short and stout, only twice as long as broad (Fig. 27).

Taeniorhynchus.—In natural waters, especially shallow trickling water forming pools, with a dense growth of *Spirogyra*, etc., in swamp water and river margins, the larvae of *Taeniorhynchus* will be readily found.

I. Note that the larva lies often embedded in the masses of green *Spirogyra* or other thread-like algae.

2. Note the great transparency of the larva and the frequency with which brilliantly green specimens are found.

Under a low objective note the following, which appear to be characteristic of this genus:—

1. The enormous horn-like and curved antennae (Fig. 29).

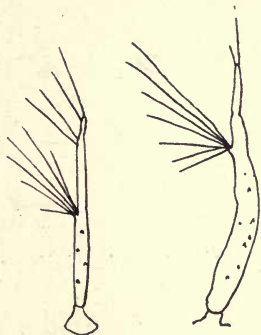


Fig. 29. *Culex fatigans* (left). *Taeniorhynchus* sp. (right)

2. The extreme length and slenderness of the syphon tube (Fig. 27).

Psorophora.—The larvae are large, half-an-inch in length. They are extremely cannibalistic.

THE NYMPH

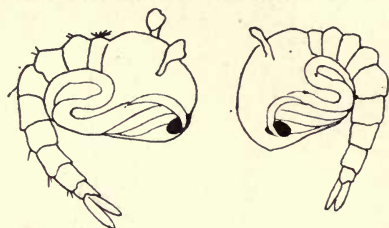
The nymphae of mosquitoes are extremely characteristic bulbous comma-shaped creatures, having a large globular body (head and thorax) and a small tail, kept more or less tucked in beneath. When disturbed they dart downwards with great speed, but very soon reappear at the surface.

Nymphs are not so easily seen in pools as larvae.

The differences in the nymphae of different

genera of the *Culicidae* are not nearly so great as in the case of the larvae.

By keeping under observation a number of nymphae, some will be seen to become less inclined for active movement, and the abdominal segments



Anopheles

Culex

Fig. 30. Nymphae of an *Anopheline* and *Culex* sp.

(tail) may be extended horizontally. Soon after these changes the adult insect emerges through a crack in the chitin of the back of the thorax. The process as seen in *Anopheles* is very fully described by Nuttall and Shipley.¹

EXAMINATION OF NYMPHAE

1. Observe that when first they appear the nymphs are light in colour, but darken very considerably later.

2. Note that just before the hatching of mosquitoes the nymph lies with the tail extended, and that silvery marks may be seen, due to air lying under the chitin.

3. Observe the emergence of the imago.

Examine the nymphs of *Anophelines*, *Culex*,

1. *Journal Hygiene*, vol. 1, part II.

Taeniorhynchus, etc., and observe that to the naked eye they are very similar.

1. Note that the nymphae of *Anophelines* lie less vertically in the water than those of *Culex*.

2. Observe that the nymphs of *Anophelines* are more elongated antero-posteriorly and compressed laterally than those of *Culex* and *Taeniorhynchus*.

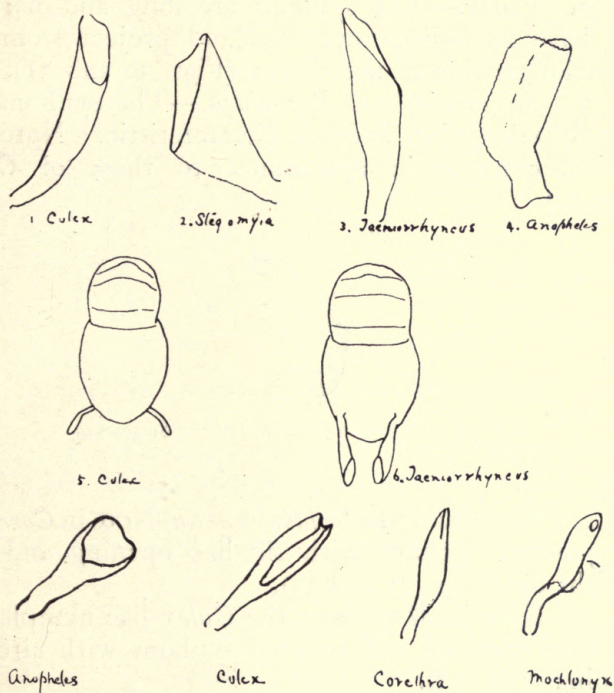


Fig. 31. Nymphal Syphon Tubes

3. Observe the very large nymphs of some common species of *Taeniorhynchus* and the great length of air syphons which are directed straight forwards in a very characteristic manner.

Place nymphae in drops of water on a slide and examine the air syphons. They appear to be of special value in the differentiation of genera.

1. *Anophelinae*.—The syphons have a square truncated end, and are proportionately much shorter than in *Culex*, and project from about the middle of the thorax (Figs. 30 and 31).

2. *Culex*.—The syphons are long and narrow, and have an oblique opening, and project from the posterior portion of the thorax (Figs. 30 and 31).

3. *Stegomyia* and *Desvoidea*.—The syphons are broadly triangular, and are characteristic. Note the marked contrast in appearance to those of *Culex* (Fig. 32).



Fig. 32. Nymphal Syphon Tube of *Stegomyia*

4. *Corethra* and *Mochlonyx*.—(a) Note in *Corethra* the pointed syphons with slit-like opening and the straight tail (Figs. 31 and 33).

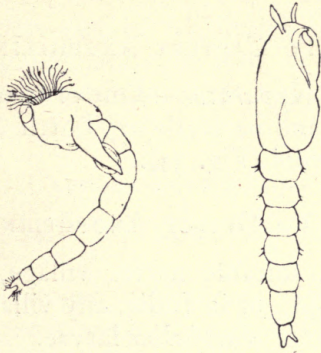
(b) Note in *Mochlonyx* the *Culex*-like nymph and the thin rounded and pointed syphons with circular opening (Fig. 31).

5. *Chironomus*.—Examine the bottoms of pools of polluted water, and note in the mud the brilliant red nymphs and larvae of *Chironomus* (Fig. 33).

1. Note that the nymph has a large globular body (head and thorax) and bears a general resemblance to mosquito nymphs.

2. Note, however, the presence of the conspicuous white feathery gills which form tufts at the side of the head.

3. Note that the nymph and larvae do not rise to the surface to breathe as do those of mosquitoes.



Chironomus

Corethra

Fig. 33. Nymphs of *Chironomus* and *Corethra*

4. Note the curious rhythmic bending movement of the larvae and nymph of *Chironomus* which, when they are present in numbers, gives the mud at the bottom of the pool a curious appearance.

LITERATURE

Miall. *Aquatic Insects*

Chapter IX

TO CAPTURE, PRESERVE ALIVE, AND EXPERIMENTALLY FEED MOSQUITOES

TO CAPTURE ANOPHELINES

Necessary Apparatus.—One or two small collecting tubes, a clean and perfectly dry bottle (whiskey bottle), some cotton wool (Fig. 34).

TO DETECT ANOPHELINES

Choose a suitable native village, *i.e.*, in Africa, any bush village; or in India, any village near a nullah or other source of *Anophele* larvae.

Determine whether *Anophelines* exist in any of the following situations:—

1. In the dark corners of sheds, cow-houses, or other out-houses.
2. Under the eaves (in darkish parts) of the huts.
3. In the huts themselves, hanging to straws, stalactites of soot, etc., etc.
4. Any other likely situations, *e.g.*, collections of dry grass; in the undergrowth in the bush (capture in this situation is difficult).

If, on inspection, none of the insects can be detected by careful scrutiny (the most concentrated attention is, as a rule, needed), the thatch should be carefully disturbed with the hand or a short stick.

TO DETECT CULEX

Examine the walls of houses, out-houses, and native huts. Examine especially clothes hung up in

native huts, old blankets, inside leather boots or boxes.

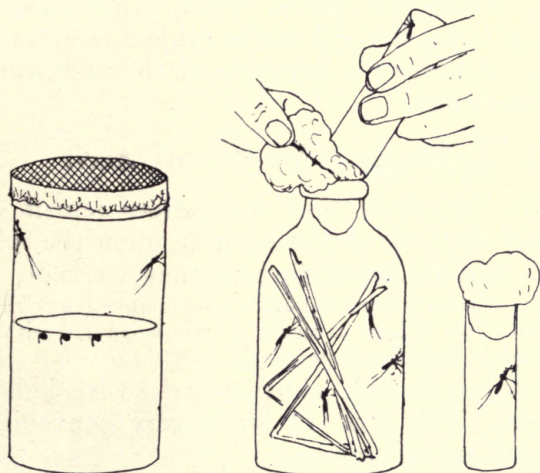
Mosquitoes seem especially fond of the smell (?) of leather.

TO DETECT TAENIORHYNCHUS

These are best caught by sitting, with a light, near a marsh or grassy land. A wall, or tent, or sheet hung up should be at hand, and kept slightly illuminated with a lamp.

TO CAPTURE MOSQUITOES

1. Place a collecting tube *very slowly* over the mosquito.
2. Insert a finger underneath, and so rapidly block the tube ; or a piece of cardboard or wool may be carefully slipped underneath.



Breeding out.

Collecting.

Fig. 34. Method of Collecting and Breeding-out Mosquitoes

3. Place a plug of cotton wool in the mouth of the tube.

4. Transfer to the large bottle by placing the tube over the mouth of the bottle and withdrawing carefully the cotton wool. Keep the bottle closed with a plug of cotton wool.

TO BREED OUT MOSQUITOES

(Fig. 34)

Collect a number of full-grown larvae and nymphae of both *Anophelines* and *Culicines*.

1. Separate the nymphae from the larvae and place them in a jar or wide-mouthed bottle half-full of water, leaving room for the insects when hatched. Cover the jar with a piece of thick cardboard or a lid, the central portion of which is replaced by mosquito netting.

2. Place the larvae where they will receive plenty of light, but will not be subject to great heat.

3. Remove the nymphae as they are seen at the end of each day.

TO KEEP MOSQUITOES ALIVE

The length of time mosquitoes remain alive in captivity depends almost entirely upon the suitability of the conditions under which they are kept.

N.B.—Except for special purposes, mosquitoes (especially *Anophelines*) should *not* be kept in cages, *i.e.*, frames covered with mosquito netting, as the mortality is immense.

Procure several 'chutney jars' with hollow glass stoppers. This form of jar is very convenient, but any other jar will serve.

Cut a piece of thick cardboard so that it will, when forced down into the jar, remain supported on the shoulders of the jar.

Fill the stopper nearly to the brim with water. Cut a thin slice of cork and place it on the surface of the water. Upon the cork place a piece of clean white paper. The paper should not quite occupy the whole of the space in the mouth of the stopper.

1. Invert the chutney jar (prepared as above) over the top of a jar in which some mosquitoes have hatched. Remove the cardboard lid and gently tap the glass. The mosquitoes will fly upwards into the chutney jar. Place it upon its stopper prepared as above.

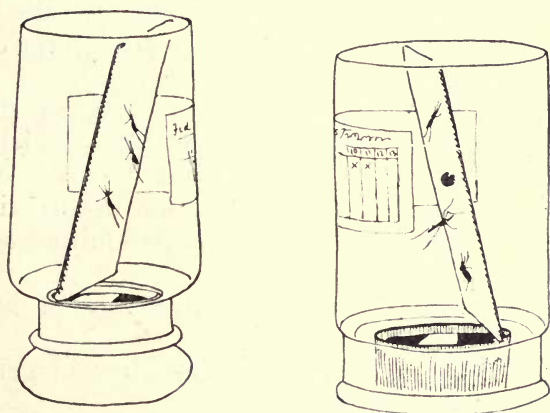


Fig. 35. Method of keeping Mosquitoes alive

Place the whole, after labelling, in a dark cupboard or other convenient place (incubator).

At the end of the first day or so, the males will be found dead upon the piece of paper, and can be removed. On the second night after hatching, most of the insects will feed, and the jar is ready for use.

2. Place the inverted jar, prepared with card-

board as above, over a bottle in which *Anophelines*, caught in a village or elsewhere, have been placed. Remove the cotton plug and shake the bottle gently to drive the insects out. Replace jar upon the prepared stopper. Place in a dark spot. Next morning remove the stopper and remove any dead mosquitoes and ova by taking out the piece of paper.

On the second night after the mosquitoes have been collected, the bottle is ready for feeding experiments. On the third day, generally, the mosquitoes have no longer any blood remaining in the mid-gut, and are ready for dissection.

The glands of any mosquitoes that may die before this may of course be dissected, if desired, on the chance of finding sporozoits.

In the use of village-caught *Anophelines*, it must be borne in mind that any subject upon which they are fed is liable to a fresh infection. In the case of natives (who sleep without hesitation in any village), the employment of village-caught mosquitoes cannot, however, be very prejudicial.

The advantages of the above way of keeping mosquitoes are :—

1. The mosquitoes will keep alive longer than in any other way known to us.
2. The immense convenience in feeding.
3. Any mosquitoes that may have died in the night can be recovered, and are not dried up.
4. It is an extremely convenient way of obtaining and examining the ova.
5. Mosquitoes which have become feeble are given the best possible chance of living, and will be found resting all day on the piece of paper.

If boxes and net-covered frames be used, an enormous mortality usually results. The dead bodies

dry up and get lost in the folds of netting, or, unless special precautions are taken, are eaten up by ants.

If jars with hollow stoppers cannot be procured, use any form of wide-mouthed jar or bottle, such as a prune jar, preserved fruit bottle, etc., and insert a piece of stout cardboard as before.

Prepare a saucer by adding a few teaspoonfuls of water and placing on this cork and paper. Invert the jar over the saucer.

TO FEED MOSQUITOES

Select a bottle in which the mosquitoes (twenty to thirty, or at least a dozen, in each bottle) are ready for feeding, *i.e.*, the second evening after hatching or collecting. Lift the bottle from the stopper, first

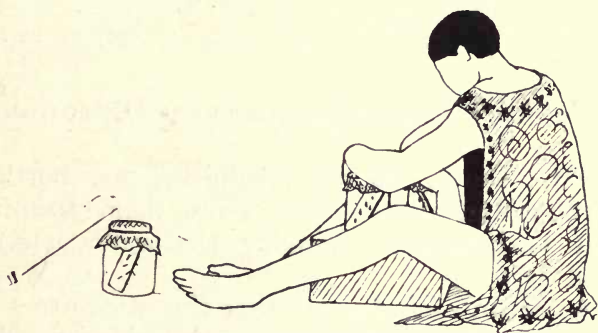


Fig. 36. Method of Feeding Mosquitoes

disturbing any mosquitoes which may be resting on the stopper, and place it mouth downwards on the table.

Slip underneath the mouth of the bottle a small piece of mosquito netting of a rather fine mesh. Tie

this around the neck of the bottle with twine. The bottle is then ready for feeding.

At, or shortly after dark, take as many bottles as may be desired to the ward or dormitory. Slightly damp the forearm (or the calf) of the patient, and, turning the bottle right side up, let the patient's arm rest upon the mouth of the bottle (Fig. 36).

In from a few minutes to half-an-hour or more the bottle will be noticed to have splashes of blood upon the bottom and sides (in the case of *Anophelines* only). If possible wait till all the *Anophelines* have fed.

Remove the bottle, invert upon a table, untie the twine, and remove the netting. Replace the bottle upon the prepared stopper.

Repeat the process every night, allowing the mosquitoes to feed by preference on the same case throughout.

Add clean water and a fresh piece of paper each time the bottle is used.

TO PREPARE FED MOSQUITOES FOR DISSECTION

After having fed the mosquitoes in a bottle for a certain number of days, allow them to remain undisturbed (merely changing the water, etc.) for several days.

Ascertain each day whether the mosquitoes have completely got rid of the blood in the mid-gut. When they are quite free from any dark colouration of the ventral aspect of the abdomen they are ready for dissection.

N.B.—If chloroform, and especially if tobacco smoke is used to kill the mosquito, it is essential to well wash the jars before again keeping mosquitoes in them.

TO FEED MOSQUITOES ON BIRDS, ETC.

1. Prepare a framework of wood and cover two sides with cardboard and two with netting. Cover one end with netting drawn tight, and to the other attach a 'sleeve' of netting. Catch or breed out a number of *Culex* (e.g., *Culex fatigans*), and place in the frame. Keep the frame in a dark place, and place a saucer of water in it.

Before placing the bird in the cage, a small bag of netting should be tied around its head, as it then remains perfectly quiet, and further, the legs may be fastened. Small birds, such as sparrows, should be carefully treated, as, otherwise, they are very liable to succumb. Pigeons should be treated in the same way, if necessary.

2. Mosquitoes may be fed singly on pigeons and other large birds by placing the end of the test tube, in which the mosquito is confined, against an area of skin free from feathers, e.g., under the wing.

FEEDING EXPERIMENTS ON BIRDS

1. Feed a number of *Culex*, e.g., *C. fatigans*, on sparrows (in which have been detected *Proteosoma* in the blood), by placing these for a time in the mosquito cage.

After feeding for one or two days, place those mosquitoes, which have fed and are gorged with blood, in a prepared jar, and keep until ready for dissection.

Note the zygotes of *Proteosoma* which generally occur in large numbers in the stomach wall, and in which very coarse and dark pigment is seen.

2. Feed some *Anophelines* on *Proteosoma* sparrows, and note that no zygotes are formed.

3. Feed some *Taeniorhynchus* on *Proteosoma* sparrows, and note the negative result.

4. Feed some *Culex* upon pigeons containing *Halteridium*, and note negative result (*Vide* p. 254).

Sparrows containing *Halteridium*, so frequently (in India) contain *Proteosoma* that, even if the latter is not observed under the microscope, it is difficult to be sure of its absence.

Chapter X

DISSECTION AND EXAMINATION OF MOSQUITOES FOR THE MALARIAL PARASITE

DISSECTION OF MOSQUITOES

Necessary Apparatus.

1. Slides and coverglasses.
2. Two needles, preferably the straight surgical needles used for making blood films, as they have a cutting edge.
3. Some salt solution, about half per cent.
4. It is convenient to have a white and a black surface for dissecting on.

Some mosquitoes are caught—by placing over the top of the jar used for feeding another empty jar of the same size—and kept alive in a dark cupboard for two or three days, until their stomachs are quite free from blood (seen by the complete disappearance of black from the ventral portion of the abdomen).

A few specimens are killed by ‘concussion,’ in a test tube.

1. Observe (if a gravid female) two whitish areas on either side of the hinder portion of the abdomen (ripening ovaries). If the blood in the stomach be not digested, a dark mass will be seen in front of these,

and possibly the extreme anterior portion of the abdomen will appear transparent (oesophageal diverticulum, containing gas) (Fig. 37).

TO DISSECT OUT THE MID-GUT (STOMACH)

1. Pull off, with forceps, the legs and wings.
2. Place the mosquito in a drop of salt solution on a slide, and place the slide on a light background.

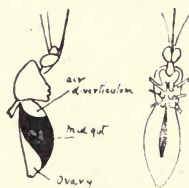


Fig. 37

Turn the mosquito upon its back and with a needle make a nick in the chitin on both sides as near the 'tail' end as possible. Now place one needle on the thorax and the other on the extreme end and pull gently.

3. After separating the segments a very short distance, remove the preparation to a dark background. Again draw apart and note the white viscera stretching between them. Make steady traction until a central rather transparent body is alone left between the two portions of abdomen.

Cut across the anterior attachments of the mid-gut.

4. Draw the body of the mosquito away from the separated segments; the mid-gut and sundry other viscera will be left attached to the latter floating in the salt solution.

Observe that when the tension is relieved, the structure last to leave the abdomen of the mosquito assumes a saccular appearance. This is the mid-gut.

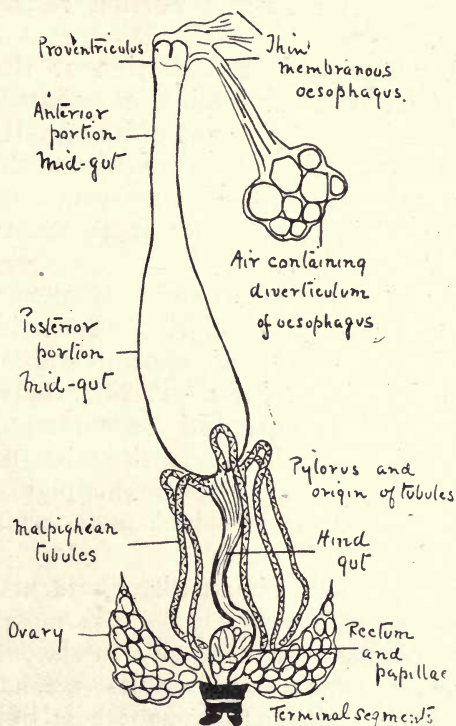


Fig. 38. Dissection of the Viscera of a Mosquito

THE VISCERA

(Fig. 38)

1. *The Ovary*.—Unless the mosquito is newly hatched, note two opaque white oval bodies (the ovaries) attached to the separated segments. If the ovaries are near maturity masses of white ova are seen.

2. *The Mid-gut*.—This extends from the level of the first pair of legs to the posterior border of the sixth abdominal segment.

(i) An anterior narrow portion resembling an oesophagus.

(ii) A posterior dilated portion at the level of the sixth (and fifth) abdominal segments in which, if the last meal of blood is not quite digested, a black mass will be seen. If any blood remains in this portion, *i.e.*, 'the stomach,' discard the specimen for one kept longer without food, as it is otherwise very difficult to see zygotes.

(iii) At the commencement of the mid-gut a ring-like, thickened portion (the proventriculus). It acts as a valve between the oesophagus and mid-gut.

3. *Malpighian tubules*.—Passing between the mid-gut and the separated segments, note five brilliantly white threads—the malpighian tubules.

4. *Hind-gut*.—Between the malpighian tubules the transparent intestine which may exhibit active peristalsis.

5. *Oesophageal diverticula*.—Attached to the proventriculus an exceedingly delicate membrane, the dilated oesophagus and three diverticula of the same, which usually contain gas bubbles. Schaudinn has shewn by adding baryta water to these bubbles that they are really carbonic acid gas. Further, also, the bacteria in these diverticula produce enzymes which are the cause of the 'irritation' of the bite, as may be shown by rubbing a diverticulum into a scratch on the skin. The salivary secretion as has been generally supposed has not this property.

The ventral diverticulum extends as far back as the fifth abdominal segment.

TO PREPARE THE MID-GUT FOR EXAMINATION

1. Cut (by pressing with the needle) across the intestine and malpighian tubes just below the termination of the saccular mid-gut. This will separate the mid-gut from the rest of the viscera.

2. Remove everything from the slide but the mid-gut. Remove excess of fluid, and see that no ova or extraneous matters are left upon the slide. Add a small drop of clean salt solution, and place a thin coverglass upon the preparation. The mid-gut will flatten out considerably. Remove with filter paper applied to the edge of the coverglass any excess of fluid. Examine under one-third inch objective and afterwards under one-twelfth.

If the mid-gut has been removed *in toto*, and the preparation not too much compressed, the following appearances are seen :—

1. The narrow anterior portion of the mid-gut with the calyx-like proventriculus at its free end.

2. If a portion of the extremely thin membrane of the true oesophagus or its diverticula be included in the preparation, it will probably be seen to exhibit peculiar markings, due probably to muscular fibres in the membrane, but resembling rather closely sporozoits. It is essential that this structure should be recognised when seen, and that the resemblance of its markings to sporozoits should not lead the beginner astray (Fig. 40).

3. The expanded posterior portion of the mid-gut. This body forms the main mass of the preparation, and is all important in relation to malarial studies.

The following appearances are seen in a good preparation :—

1. Well-defined tubes with spiral lining (air

tubes or tracheae). Note that these branch and ramify upon the surface of the mid-gut and malpighian tubes (Fig. 39).

2. Large muscular fibres, together with elastic fibres, forming a meshwork. Note that they are circular and longitudinal (external). Note that at the edge of the viscus they are seen in optical section (Fig. 39).

3. Large cells with large nuclei and granular protoplasm (epithelium of mid-gut). Note some in

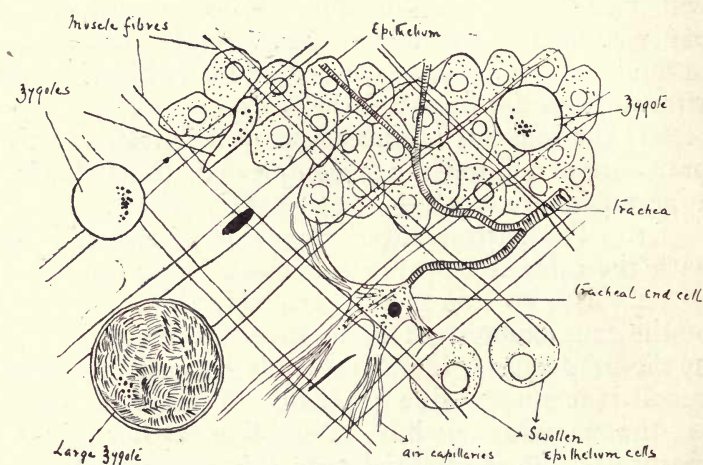


Fig. 39. Microscopic appearance of Mid-gut, shewing Cell Structure and Zygotes

situ forming a single layer of polygonal epithelium, and others detached and in process of being carried along by fluid streaming from interior of mid-gut. Note that in some places these cells are undergoing vacuolisation with dancing of the protoplasm granules (Fig. 39).



Fig. 1



Fig. 2



Fig. 3

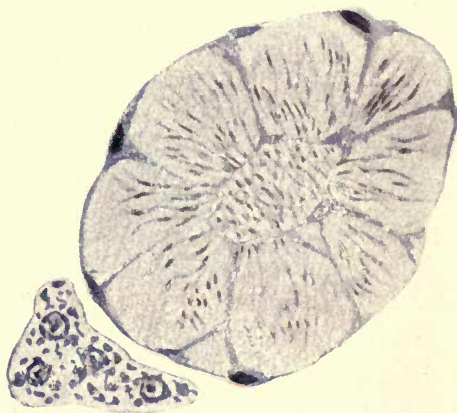


Fig. 4



Fig. 5

PLATE II

Fig. 1.—Young zygotes of the benign tertian parasite.

Fig. 2.—Young zygotes of the malignant tertian parasite.

Fig. 3.—Mature zygote, outside epithelium of mid-gut (Section, haematein).

Fig. 4.—Transverse section of salivary gland shewing sporozoites *in situ* (haematein).

Fig. 5.—Sporozoites from the salivary gland (Romanowsky stain).

4. Note any contents of the stomach—

- (i) Remains of blood.
- (ii) Crystals of various kinds.
- (iii) Gregarines, flagellates, bacteria, etc.

5. Note that in focussing downwards one passes through a double thickness of wall. Note that the air tubes are focussed on the upper and lower surfaces of the preparation, and the epithelium and crystals in the middle.

6. Trace several of the finer air tubes to their apparent termination, and note that when they lose their spiral lining they are continued as very fine transparent tubules (air capillaries). Note that at the point of breaking up, one can generally make out large stellate cells (tracheal cells) (Fig. 39).

7. Observe in some preparations, large oval cells of brownish colour lying upon the outer surface of the stomach. Note that they are rather opaque, and contain a certain amount of diffuse yellowish pigment. They are so-called pericardial cells (Fig. 39).

8. Observe, in most preparations, one or more large clear cells with a small nucleus, and filled with oil globules (cells of the fat body) (Fig. 39). These lie upon the stomach and, in common with the last-named cells, are accidental in this situation.

THE EXAMINATION OF THE MID-GUT FOR THE ZYGOTE OR OOCYST STAGE OF THE MALARIAL PARASITE

(The examination of the stomach blood for flagellating and the motile or vermicule forms is deferred to a later Chapter).

Obtain a number of *Anophelines* (not *M. rossi*) from some native hut, or better, those specially fed. Keep these alive for two or three days until no blood

remains in the mid-gut (for methods of keeping alive see p. 90).

Prepare the mid-gut as described above. A considerable number may prove negative, but a variable percentage will be positive. Examine with one-twelfth inch.

Carefully note the presence of small collections of pigment of the nature of *malarial pigment*. By careful focussing, the younger forms may be seen as clear oval or round bodies, $6-7\mu$, in which the distinct clearly defined pigment occurs. The more advanced forms can scarcely be missed. It is necessary to bear in mind the normal structures and the fact that, until the parasite reaches a considerable size and has a very sharply defined cyst wall, pigment, of the character belonging to the species of parasite concerned, is present.

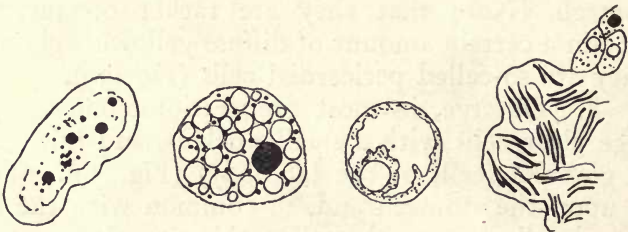


Fig. 40. (Left to right) Pericardial Cell, Fat-Body Cell, Swollen Epithelium Cell, Diverticulum shewing Sporozoit-like appearance

1. Zygotes of malignant tertian shew, when young, a clump of pigment resembling black pepper grains (Fig. 41).

2. Zygotes of simple tertian shew yellowish or golden pigment in wisps (Fig. 41).

3. Zygotes of quartan shew rather coarse pigment in a clump (Fig. 41).

The older zygotes ($40-60\mu$) are indistinguishable

as regards the species of parasite concerned. They may shew :—

1. A very clear and distinct oocyst wall (adventitious).
2. The formation of sporoblasts.
3. In still more developed forms, the sporoblasts are seen to be surrounded by a radiating arrangement of young sporozoites or blasts (Figs. 14 and 39).
4. Fully developed forms are large cysts packed with many hundreds of fine sickle-shaped bodies, and if they are ruptured, these latter escape into the surrounding fluid, and are readily distinguished with a sixth-inch lens as sporozoites (Fig. 45).

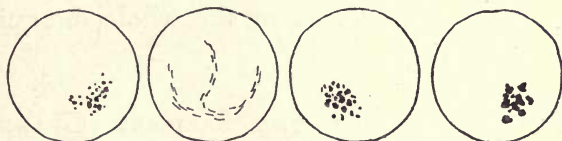


Fig. 41. (Left to right) Zygotes of *Malignant Tertian*, *Simple Tertian*, *Quartan*, and *Proteosoma*

TO MAKE PERMANENT PREPARATIONS OF ZYGOTES

Method 1.—In case of a specimen shewing zygotes, place a large drop of ten per cent. formalin on one side of the coverglass, and draw this through by filter paper placed on the other side. Repeat several times. Remove excess of the formalin with filter paper. Seal edges of coverglass first with glycerine jelly and then with cement (p. 408).

The zygotes will retain their appearance as seen in the fresh specimen.

Method 2.—Run formalin through as in Method 1.

When an excess of fluid is present, slide off the coverglass. The flattened mid-gut will probably remain attached to the coverglass.

Wash in water, and stain lightly with methylene blue. Wash in water and allow to dry. Warm gently to ensure complete dryness, and place the coverglass, mid-gut downwards, upon a drop of balsam upon a slide.

The muscular fibres and other structures of the mid-gut will be well exhibited. The zygotes will be stained rather a dark blue. If not too darkly stained, the pigment of the zygotes will have the appearance it had in the fresh specimen.

Method 3.—For more minute histological examination, imbed the stomachs, or the whole mosquito, in paraffin (p. 46).

TO DISSECT OUT THE SALIVARY GLANDS

This is quite a simple proceeding if it be remembered where they lie. They are intra-thoracic structures, and they commence at the hinder portion of the neck and end opposite the first pair of legs. They lie ventrally, in fact, roughly speaking, they lie just above the origin of the first pair of legs (Fig. 42).

The simplest and most rapid method, and the one that hardly ever fails, is the following :—

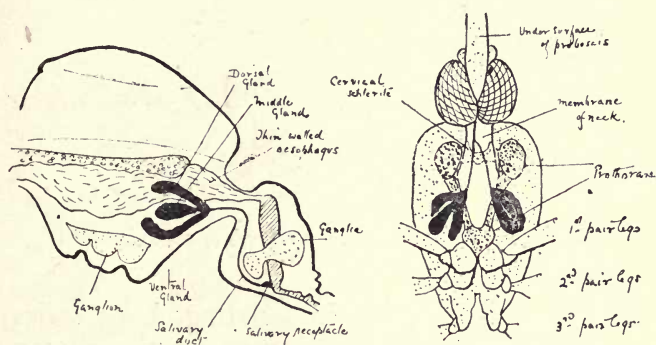
1. Place the mosquito in a drop of salt solution on its right side, with the head pointing towards you, as you dissect.

2. Place the needle of the left hand on the thorax to steady it, and place the needle of the right hand on the back of the head and make steady *gentle* traction.

3. If done carefully, it will be seen that the head has pulled out a little mass of white tissue from the

thorax (the dissection is best done on a dull-black surface).

4. Examine the piece of tissue under a half-inch lens. (The diaphragm should be as nearly closed as possible). The glands will be seen hanging on to the neck as finger-like, transparent, *glistening* bodies. Muscle has a greyish look, and even the fat body is not so refractive as the glands.



Showing position of Salivary Glands

Fig. 42. Showing position of Salivary Glands

5. Now place one needle on the head, and with the other make a transverse cut between the head and the attached portion of the glands.

6. Examine again the now separated glands. Generally all six are with certainty got in this way.

7. If the glands are not found on the neck, proceed with the dissection by Method 2.

8. When dissected out in this way, they are generally quite free from surrounding tissues, but if found necessary they can be teased out further and placed in fresh drops of salt solution.

9. At all stages of the dissection make sure that the glands are really present and that they have not floated to the side or stuck to the needles.

10. By this certain and rapid method, as many as one hundred glands may be dissected out, put under a coverglass, and examined microscopically in a day's work.

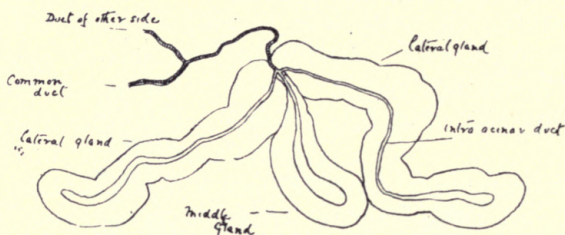


Fig. 43. The Salivary Glands of one side

Method 2.—Consists in isolating, by a series of cuts, the anterior ventral portion of the thorax in which the glands lie.

1. Make a cut obliquely in an antero-posterior direction, so as to sever the main mass of thoracic muscle.

2. Make a cut at right angles to this, passing just behind the attachment of the first pair of legs.

3. Cut through the neck.

4. The glands lie in the portion thus isolated. Considerable teasing out is still required to isolate them from the surrounding tissues. Examine each portion of tissue separated out, and remove to a fresh clear drop of salt solution.

Remember that in examining under the microscope the apparent right hand is really the left, and *vice versa*.

This method, which is longer than Method 1, requires more dissecting and teasing out in order to isolate the glands cleanly, and, as we have said, may still be followed, even if No. 1 has failed; but our experience has been that Method 1 is learned at once without any difficulty.

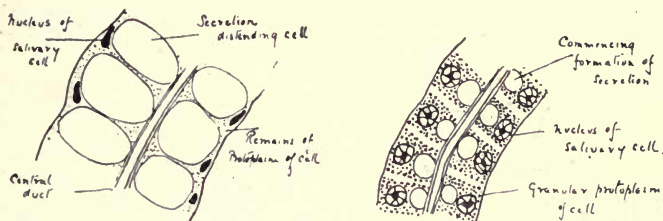


Fig. 44. Microscopic Structure of Salivary Acinus and that of a Newly-Hatched Mosquito (right)

Ascertain that the glands of either side consist of three acini, the ducts of which join almost immediately after leaving the acinus to form a single long duct.

1. Observe that of the three glands of each side (Figs. 43 and 46):—

(i) Two are highly refractive, and the cells in these are very distinct and clearly defined (lateral glands).

(ii) One is much less refractive, and the component cells are much less easily defined (central gland).

2. Observe that each acinus has a duct running through its whole length, and that the secretory cells form a single row around this.

3. Observe that each secretory cell has a large mass of clear secretion within it, forming the chief bulk of the cell, and that the nucleus is flattened and pushed to the periphery (Fig. 44). Pressure tends to

force the secretion out of the cell in viscid looking droplets. The secretion of the lateral glands is far more refractive than that of the central (Fig. 46).

4. Ascertain that the duct formed by the junction of the three intra-acinar ducts joins, eventually, the similar duct from the other side, to form a

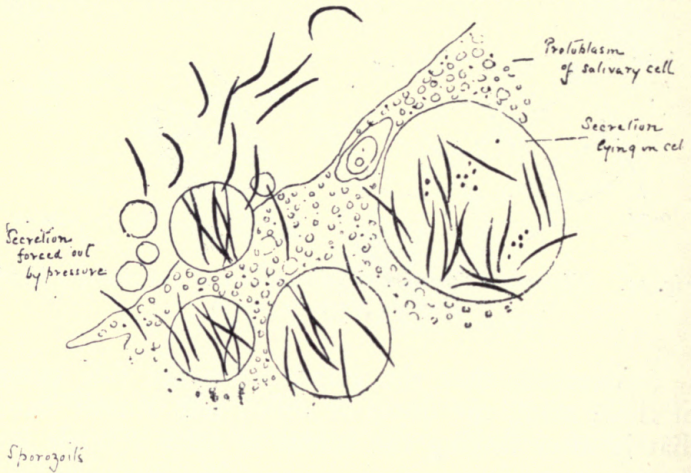


Fig. 45. Sporozoites in the Salivary Gland
(Fresh preparation)

common salivary duct which passes into the salivary receptacle. The duct is thick walled, and is lined with a spiral thread resembling that of a tracheal tube.

EXAMINATION OF THE SPOROZOIT FORM OF THE MALARIAL PARASITE

Obtain a number of *Anophelines* (not *M. rossi*) from a native quarter (five per cent. to twenty per cent. or more have sporozoites in the glands), or *Anophelines* fed for twelve days or more at a temperature of

80° F. Prepare specimens of the glands, as described above. Press with the point a needle on the coverglass, so that the gland is ruptured, and the secretion poured out as droplets into the surrounding fluid.

Examine with one-sixth inch. If sporozoits are present they are generally very numerous, and large numbers of fine, very distinct curved rods, will be easily seen with this power, lying throughout the fluid around the gland and packed in large numbers in the substance of the gland. Finally, examine with one-twelfth inch (Fig. 45).

The sporozoits have a mean length of 14μ , and vary between 10μ and 20μ , and are $1-2\mu$ in width.

Examination of Motion of Sporozoits.—Dissect out the glands and, when isolated cleanly, transfer to a drop of human serum, previously got ready by allowing blood to clot in a small tube. Three kinds of motion may be observed :—

1. Formation of curves.
2. Formation of ring-formed contractions.
3. Locomotion. Forward motion.

Penetration of Red Cell by Sporozoits.—This has not been seen in case of sporozoits of the salivary glands, but has been observed twice by Schaudinn in the case of sporozoits from a ruptured cyst in the stomach. Repeat the observation by mixing a little blood with sporozoits under a coverglass.

To Prepare Permanent Preparations of Sporozoits.—Pressing firmly upon the coverglass, draw it along the slide, so that a film is made on coverglass and slide.

Dry by rapidly waving the slide and the coverglass in the air. Fix both in alcohol, and stain with Romanowsky. Wash, dry, and examine without coverglass with an oil immersion.

The sporozoits appear as fusiform bodies with a

central red mass of chromatin. They are about 14μ in length, with one end often more pointed than the other.

Wash off the oil with xylol, dry and label.

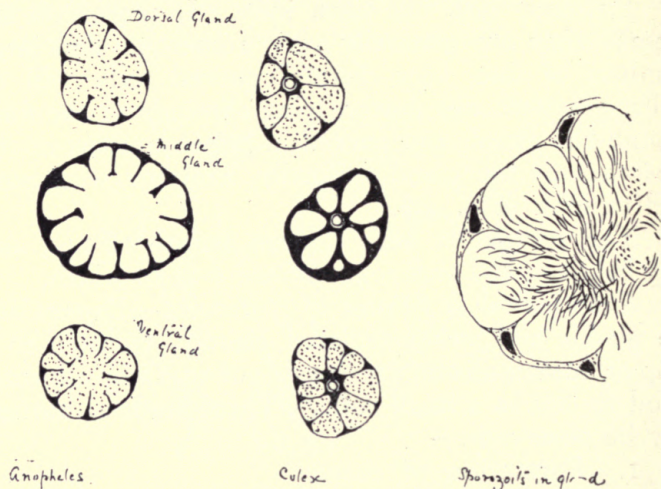


Fig. 46. Sections of Salivary Glands, shewing differences between the Middle and Lateral Glands and between those of *Anopheles* and *Culex*, also Sporozoites in the Glands of an *Anopheles*

THE REPRODUCTIVE SYSTEM

To Examine the Spermatheca.—With the needle cut off the extreme tip of the abdomen—the last or eighth segment only. Place this in a very small drop of salt solution, and tease the fragment carefully apart. A small pea-like body (in *Culex*, three) will be seen. Isolate this (the spermatheca) as much as possible from other tissue and cover with a coverglass (Fig. 47).

Spermatozoa.—Observe, under a low power, the brown chitinous spermatheca and its duct. Press

firmly on the coverglass so as to rupture the spermatheca. Examine under one-twelfth inch.

Observe the masses of fine hair-like actively motile bodies, if (as is probably the case) the mosquito has been fertilised. Isolate some of these; they possess the characters of spermatozoa (Fig. 47).

Examine the spermatheca of a mosquito newly hatched; it does not contain spermatozoa.

TO EXAMINE THE OVARIES

Observe that when the ovaries nearly reach maturity they are readily detected as white areas on either side of the posterior part of the abdomen, and

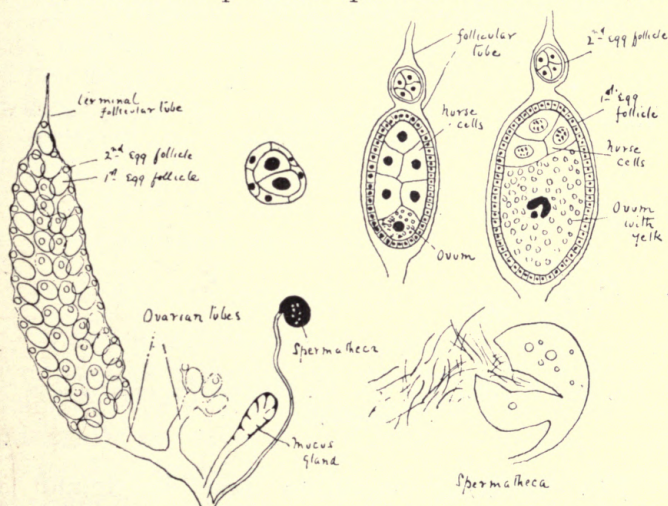


Fig. 47. Spermatheca with Spermatozoa. Structure of Ovaries

that when fully developed they occupy the whole of the lateral and dorsal portions of this.

Drag off the last few segments of the abdomen in a drop of salt solution, and allow the ovaries to float

out in this. Observe that they are pyriform bodies, the apex being above (Fig. 47).

Ascertain that each ovary consists of a large number of follicular tubes, commencing as fine threads and ending in the oviduct. Observe especially the follicular tube forming the apex of the ovary, as here this is most readily made out.

Ascertain that each follicular tube contains several egg follicles, the lowest of which is the most advanced in development.

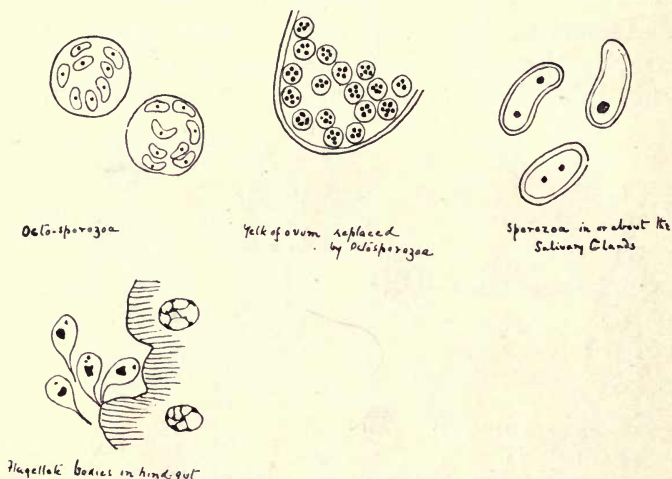


Fig. 48. Protozoa other than the Malarial Parasite found in *Anophelines*

The following organisms may be found in mosquitoes apart from the various stages of the malarial parasite :—

1. *Encysted Trematodes*.—Mostly found in the tissues, near the neck ; also free in the stomach.

2. *Nematodes*.—In the thorax or abdominal cavity.

3. *Sporozoa*. — (a) Sausage-shaped bodies in masses, sometimes in close connexion with the salivary glands (Fig. 48).

(b) Octosporozoa, consisting of eight small sausage-shaped bodies in small cysts, enormous numbers of which replace the yolk of the ovum (Fig. 48).

(c) *Gregarines*.—Occasionally on the outside of the stomach or encysted in the malpighian tubes. In the larvae, the worm-like gregarine will be found actively motile in the malpighian tubules.

4. *Flagellates*.—In the rectum and hind-gut, frequently in enormous numbers, and shewing, when stained, large numbers of developmental forms (p. 362).

5. *Developing Embryos of Filaria*.—These are seen as sausage-shaped bodies with a terminal spike in the dissection of the salivary glands. In sections of mosquitoes they are seen in the muscles, especially in those of the thorax, and the developed embryos occur in the labium or about the base of the neck.

6. *Micro-organisms and Sporozoa (Nosema)?*—In the oesophageal diverticula.

TO CUT SECTIONS OF MOSQUITOES

The following combined celloidin-paraffin method gives admirable results where it is required to preserve intact, especially *the chitinous parts*, and by this method and hardly any other it is possible to procure sections of a hard proboscis like that, for instance, of *Stomoxys calcitrans*.

A difficulty that occurs with chitinous organisms like flies, is the penetration of the fixative. So difficult is it to get perfect fixation that it is advisable where details of structure are being studied to remove the part required and to fix by itself.

1. Select *Anophelines* free from blood and kill

by chloroform vapour or by 'concussion' in a test tube.

2. Fix in (a) acetic-alcohol for five to ten minutes (glacial acetic acid one part, absolute alcohol six parts, chloroform three parts), wash in absolute alcohol till free from acid. (It is often advisable, especially with thick flies, to nick the chitin at some unessential part in order to allow penetration of the fixative.) Or (b) sublimate-alcohol (p. 404), or (c) absolute alcohol, fifteen minutes.

3. Place in one per cent. celloidin for a day and in three per cent. celloidin for another day. These times may with advantage be increased, but sometimes a few hours only suffice. (The solution of celloidin is made by dissolving dry chips in 50 c.c. alcohol and then in a day or so adding 50 c.c. ether.)

4. Transfer to cedar-wood oil, and when the mosquito sinks transfer to—

5. Paraffin for half to one hour and then proceed as before (p. 46).

THE EXAMINATION OF SEPARATE ORGANS

1. Remove the mid-gut or the ovaries or the salivary glands and fix in sublimate alcohol or Zenker (ch. xxxiv), and proceed as on p. 46.

2. The salivary glands can thus be studied by embedding only the head and thorax; zygotes by embedding only the abdomen or the dissected-out gut.

The times for the dissected organs in various fluids is very short, only five to ten minutes in each fluid.

3. Stain with haematein (p. 408).

LITERATURE

Grassi: *The studies of a Zoologist* (in German or Italian).

Chapter XI

INTERNAL ANATOMY OF MOSQUITOES

THE ALIMENTARY CANAL

The alimentary canal is specialized on account of the blood-sucking habits of the mosquito. It differs from many insects in not possessing any caecal diverticula of the mid-gut. It also differs in the possession of five malpighian tubules, these being in insects usually even in number (Fig. 38).

The parts of the alimentary canal are as follows :—

The mouth	}	The fore-gut.
The pharynx with pumping organ		
The oesophagus		
The oesophageal diverticula	}	The mid-gut.
The homologue of the proventriculus		
The stomach (so-called)		
The pylorus		
The pyloric dilatation	}	The hind-gut.
The ileum		
The colon		
The rectum with rectal papillae		

The mouth, pharynx, and oesophagus are ectodermal in origin, and both the mouth and pharynx are lined with chitin. The hind-gut is also ectodermal in origin; it does not possess, however, any portion lined with chitin. The mid-gut is the true digestive portion of the tract.

The Pharynx.—The pharynx, which is lined throughout its extent with chitin, passes upwards and backwards through the ganglionic ring formed by the supra and infra-oesophageal ganglia and their commissures. At first it is narrow, but posteriorly becomes a large chamber (the pumping organ) (Fig. 52).

The pumping organ occupies with its muscles a large portion of the head behind the level of the cerebral ganglia. In the state of rest its lumen is triradiate in transverse section. The walls are formed of three large and thick chitinous plates, one placed on either side, and one superiorly. Into each of these plates powerful muscles are inserted. The plates are connected by thin non-chitinous membrane, and their edges are rolled so that they form a spring capable of returning to their original position so soon as the separating force of the muscles ceases.

Posteriorly, where the pharynx becomes very narrow, a sharp bend occurs and a valvular action is produced. The whole forms a very powerful suctorial apparatus.

The Oesophagus.—Immediately beyond the pumping organ the chitinous layer ceases, and the rest of the fore-gut is formed of excessively thin membrane. At the junction of the two portions a sharp bend occurs, and the floor projects so as to form a valvular flap.

The thin-walled oesophagus is a large dilated sac, whose walls are supported by surrounding structures. Into the posterior wall of the dilated and thin-walled oesophagus projects the papilla-like anterior portion of the mid-gut.

The Diverticula of the Oesophagus.—From the oesophagus two or three diverticula, similar in nature to the oesophagus, extend backwards. Of these, one is of great size, and usually contains gas bubbles.

This usually extends into the abdomen, and is a prominent object in dissections and sections (Fig. 38).

The Homologue of the Proventriculus.—There is no true proventriculus as in many insects. There is, however, an interesting fold of the fore-gut into the mid-gut which represents this organ. The muscular bundles are here increased, and the whole forms a valvular muscular organ (Fig. 38).

The Mechanism of Feeding.—The powerful pumping action which must result from a drawing asunder of the three large chitinous plates of the pumping organ is very evident. These plates, also, when drawn apart must, by reason of their spring-like shape, revert to their original positions close together, without any muscular aid. Posteriorly the valve-like arrangement mentioned before prevents regurgitation. Further, when the blood reaches the junction of the oesophagus and mid-gut the invaginated portion is withdrawn, and is distended by the entering blood into a distinct 'crop,' the valvular function is suspended, and the blood flows onward.

The Mid-gut.—The mid-gut extends from the proventriculus to the origin of the malpighian tubes.

The anterior narrow portion of the mid-gut lies in the thorax, and does not become distended with blood. The posterior portion when fully dilated fills the greater portion of the abdomen, the viscera being pushed into the last few segments.

The Hind-gut.—The hind-gut is short and passes in one or two bends from the pylorus to the anus. Immediately beyond the pylorus there is a considerable dilatation which is poorly supplied with muscular fibres: into this open the five malpighian tubules. For a short distance beyond this the lumen is narrow (ileum), but

becomes gradually larger (colon). At the termination of the colon there is a slight constriction, after which the canal dilates again to form the rectum (Fig. 38).

Into the rectum project six solid growths, the so-called rectal glands, which are, however, papillae. Posteriorly the rectum ends in the anus close above the genital canal.

The appendages of the alimentary canal are:—

The Salivary Glands.—The salivary glands consist of six tubular acini lying three upon either side. A duct can be seen traversing almost the entire length of each acinus. Shortly after leaving the acinus, the three unite to form a single duct. Beneath, and in contact with the lower surface of the suboesophageal ganglion, the ducts of each side unite to form a common salivary duct which passes forwards and enters the chitinous first portion of the alimentary canal close to the base of the proboscis (Fig. 42).

The Malpighian Tubules.—These are five in number and open into the first portion of the hind-gut immediately beyond the pylorus. Their blind ends are held in position in the neighbourhood of the rectum by tracheal branches. They pass forwards in loops above their origin, so that, in transverse section, as many as ten may be seen cut across.

The Vascular System.—As in most insects where the respiratory system ramifies throughout the whole body, the vascular system is not well developed. A dorsal vessel or heart and an anterior prolongation of this (aorta) are the only closed blood-vessels. Apart from the dorsal vessel the blood circulates in large blood spaces, which lie between the lobes of the fat-body and among the muscles and viscera.

The dorsal vessel passes close beneath the tergal plates throughout the abdomen. It is very thin walled,

and is not provided with valves. The upper portion is attached to the dorsum at intervals by suspensory fibres (muscular), so that a festooned appearance is given in longitudinal section. There is, however, no true division into compartments. Laterally large cells (pericardial cells) are arranged throughout its entire extent, and fibres of a muscular nature (alary muscle) pass from the body wall and end in branches in close connexion with the dorsal vessel.

At the first abdominal segment the dorsal vessel dips down beneath the mesophragma, lying as it does so in direct contact with the cuticle. In the thorax it again arches upwards, and lies between the lower portions of the antero-posterior wing muscles close above the anterior portion of the mid-gut.

In the anterior third of the thorax it divides into two smaller portions which pass outwards, and coming in contact with the salivary ducts enter the neck.

Blood spaces without definite walls occur throughout the body. The thorax especially contains large spaces among the muscles, and the complex fat-body which lies between and supports the organ is everywhere bathed with blood fluid.

THE REPRODUCTIVE SYSTEM

The organs of the reproductive system are :—

1. Ovaries.
2. Oviducts and common oviduct.
3. Mucus gland and duct.
4. Spermathecae and ducts.

The ovaries occupy a variable position dependent upon the state of their development. In the newly-hatched mosquito they are small bodies lying in the

fourth and fifth abdominal segments close by the posterior portion of the mid-gut, hind-gut, and malpighian tubes towards the venter, so that eventually the ovaries occupy nearly the whole of the posterior portion of the abdomen. Each ovary consists of very many follicular tubes, each containing egg follicles in different stages of development. In the mature ovary the lower follicles have in every tube become the large completely-formed egg (Fig. 47).

The oviducts are muscular tubes passing from the ovaries. They join beneath the rectum to form the common oviduct, which is still more abundantly supplied with muscle fibres, and which eventually opens beneath the anus.

The spermatheca is a chitinous sac, which in the impregnated female is filled with a mass of spermatozoa. Its duct is long and twisted and opens into the common oviduct near its termination. (In *Culex spp.* there are three spermathecae.)

The mucus gland, globular or ovoid in shape, opens by a short duct into the same region.

The Fat-body.—The adipose tissue is disposed in two ways.

1. As a general lining to the body wall, being nearly everywhere present directly beneath the cuticle, and

2. As lobular masses lying in among the organs and muscles. Thus a large pad lies over the compound thoracic ganglion, and sends processes which lie in among the salivary glands and other viscera. Other smaller masses lie in the head and abdomen.

HISTOLOGY

Methods.—The examination of the fresh tissues frequently reveals structures not easily seen in fixed

preparations. The tissues are best dissected out in saline of low tonicity, 0·3 or 0·4 per cent., as insect juices have a lower isotonic point than those of mammals.

THE HISTOLOGY OF THE ALIMENTARY CANAL AND APPENDAGES

The epithelial lining differs considerably in the mid-gut from either the fore-gut or hind-gut. In the mid-gut the possession of a marked striated border by the epithelial cells is characteristic. The muscular fibres of the alimentary canal are striated throughout.

The Fore-gut.—The anterior portion of the fore-gut is lined by chitin and does not differ from the cuticle in structure. It consists of a single layer of cubical cells of small size. The oesophageal dilatation and its diverticula resemble one another in structure. In the adult mosquito they consist of an extremely delicate membrane formed of a single layer of flattened cells, with externally some scattered muscular fibres. In fresh preparations peculiar wrinklins of this membrane are seen, which may appear like bundles of sporozoits. A similar appearance is seen in the dilated portion of the hind-gut just beyond the pylorus.

In the majority of mosquitoes the walls of the oesophageal diverticulum are crowded with micro-organisms and bodies which appear to be protozoal in nature.

The Mid-gut.—The epithelium consists of a single layer of large cells, which are columnar in the undistended organ, but become flat and pavement-like when the organ is full of blood. They have a finely-reticulated protoplasm, which stains more deeply towards the free border. Stained with Heidenhain's

haematoxylin, alcohol-hardened specimens are seen to contain numerous stained granules, collected especially in the outer portion of the cell. These are especially abundant in the anterior portion of the mid-gut. They have also, very frequently, a number of small clear vacuoles (droplets), which become more frequent and of larger size towards the free border of the cell. The most marked feature of the cell is the clear striated border which is present in all the cells of the mid-gut, but absent in all other portions of the alimentary canal. The striated border is best marked in the undistended organ, and becomes almost invisible in the fully distended state, when the cells are much flattened.

The nucleus of these cells is large and centrally situated.

The muscular coat is very thin. It consists of an open mesh-work of long muscular fibres running longitudinally and circularly.

The individual muscle fibres are very long, fusiform, striated fibres. On the outer surface of the mid-gut lie numerous large branched cells in which the small tracheae end, and from which bundles of minute structureless air tubes pass into the wall of the mid-gut. These cells are frequently well shown in gold chloride specimens. Similar cells occur throughout the viscera in connexion with the tracheal endings.

The Hind-gut.—Structurally the small and large intestine are similar, whilst the dilatation beyond the pylorus, and especially the rectum, differ from these.

The dilatation which occurs at the origin of the malpighian tubules is thin-walled and poorly supplied with muscle fibres. The cells lining it are small and flattened.

The intestine is lined with a single layer of large cubical cells; external to these is a muscular coat.

The cells of the intestine have large nuclei. The protoplasm is finely reticular, and stains less deeply than that of the cells of the mid-gut. Stained with Heidenhain's haematoxylin, no granules are present as in the cells of the mid-gut. They have no striated border.

In the rectum the cells become small and flattened. There are here, however, bodies usually termed rectal glands. These are papillae covered with a single layer of much hypertrophied cells resembling those lining the small intestine and colon.

The Salivary Glands.—The salivary acini lie in a cleft in the fat-body, which latter comes in close contact with the glands. Each gland acinus consists of a single layer of large cells, limited externally by a delicate sheath (basement membrane) and internally by the intra-glandular duct wall.

In *Anophelines* the intra-glandular duct becomes larger as it approaches the termination of the acinus, and forms a large cavity.

In *Culicines* the duct remains of the same diameter throughout the acinus, and terminates abruptly near the end of the acinus without any dilatation.

In both *Culicines* and *Anophelines* there are two types of gland acinus. These are recognizable both in the fresh gland and in fixed specimens. From their appearance in the latter they may be termed

(1) The granular type.

(2) The clear or colloid-like type.

The Granular Type.—The greater portion of the acinus consists of cells whose nucleus and protoplasm has been pushed to the outer portion of the cell by a large mass of secretion which occupies almost the whole of the cell. In the fresh gland this secretion appears as a clear, refractive substance, and can, by

pressure, be made to exude from the cell in refractive globules. In specimens hardened in alcohol, this clear secretion appears as a granular mass, occupying the greater portion of the cell. It stains faintly with haematein, and shows under high powers a coarse reticulum and isolated globules, an appearance probably due to the precipitation or coagulation of the secretion by the alcohol.

The protoplasm of the cell occupies, in the fully-matured gland, only the extreme periphery, and the nucleus, which is much degenerated, is pushed to the outer portion of the cell, and usually lies in the angular interval left at the base of two or more contiguous cells.

The Clear or Colloid-like Type.—Of this type there is but a single acinus upon either side, which usually lies between the two acini of granular type (Fig. 46).

In the fresh gland the cell outlines are not so distinct as in the granular type, and the secretion, when extended by pressure, is much less refractive. In alcohol-hardened specimens, the acinar cells contain a large mass of clear, homogeneous secretion which, as in the last-mentioned type, fills almost the entire cell, and pushes the protoplasm and nucleus to the periphery.

In the clear type, however, the protoplasm is always in greater amount than is the case with the granular type, and the nucleus never becomes so greatly degenerated. The clear, homogeneous secretion stains readily with haematein, and may even stain quite deeply. With Heidenhain's haematoxylin it frequently becomes almost black. It resembles very much in appearance colloid substance as it is seen in the mammalian thyroid.

In *Anophelines* this substance also distends the central duct space within the acinus. In this situation an appearance is sometimes produced which resembles faintly-stained sporozoits, but which is a normal condition.

The Malpighian Tubules.—The malpighian tubules are tubular bodies with caecal ends, which open into the hind-gut. The cells are extremely large, being, next to the pericardial cells, the largest in the body. Each cell contains a large nucleus, and contains numerous large granules which stain feebly with haematein, but powerfully with Heidenhain's haematoxylin. Numerous fatty granules are also present. Each cell is wrapped round a central lumen, the cells being arranged alternately, so that a zig-zag appearance is given in section. The inner portion of each cell is markedly striated, the lumen being thus bounded by a striated area. In relation with these tubules, a large number of tracheae and tracheal end-cells exist.

In certain conditions the malpighian tubule cells may be found quite free from granules, though otherwise unchanged. This change occurs in mosquitoes with large numbers of flagellates in the rectum and hind-gut.

The Vascular System.—The dorsal vessel is a delicate-walled tube composed of longitudinal and oblique fibres with a nucleated inner layer. The fibres may be traced directly from the terminations of the branched alary muscle fibres. The alary fibres break up into fibres which pass in close connexion with the large pericardial cells, and eventually form (1) fibres passing into the dorsal vessel as longitudinal fibres, (2) fibres joining in an anastomosis in connexion with the floor of the dorsal vessel.

The pericardial cells are extremely large cells lying on either side of the dorsal vessel throughout its whole extent. They are by far the largest cells in the mosquito, varying from 30μ to 50μ in longitudinal diameter. They are elongate or pear-shape in form, and contain several nuclei. The nuclei usually show signs of degeneration. The peripheral portion of the cell stains more deeply than the central portion, which contains the nuclei and small stained granules. There is a considerable number of masses of a light yellowish pigment resembling that found in the large visceral ganglia cells. The fibres from the branches of the alary muscles pass over and around the pericardial cells to reach the dorsal vessel. From their structure and situation the pericardial cells appear to be of the nature of ganglion cells (Fig. 40).

The Fat-body.—The fat-body, both where it occurs as a portion of the body wall and where it lies as free lobulated masses, consists of cells containing numerous oil globules. The cells are of considerable size, and their borders may be frequently traced as polygonal areas. The nuclei are oval in shape with a central mass of chromatin and chromatin threads. Besides oil globules the cells contain granules staining with haematein, and minute droplets of a highly refractive, dark substance, which gives the appearance of pigment. These droplets are larger in amount in old mosquitoes than in those freshly hatched (Fig. 40).

The Reproductive System.—Each ovary consists of a large number of follicular tubes whose lower ends open into the ovarian tube, and whose upper ends terminate in a delicate supporting filament (terminal filament). The apex of the ovary is formed of a single follicular tube, whose filament is attached to the fat-body of the fourth segment.

Around the whole ovary there is a delicate nucleated sheath.

Each follicular tube contains one or more egg-follicles in different stages of development. In the freshly-hatched mosquito each follicular tube contains an undeveloped egg-follicle. As this develops, a second and a third undeveloped follicle appear above it, which again undergo development into mature eggs. The follicle at first consists of two to four large cells, with large nuclei surrounded by a single layer of smaller epithelial cells (Fig. 47).

The central cells then increase in size and number, so that many very large cells are contained in the now enlarged follicle. The surrounding epithelial cells also become larger, and rapidly increase in number so as to form a layer of regular cubical cells surrounding the follicle. The central cell nearest the ovarian tube is the ovum, the rest are nurse cells, and eventually disappear. Both the ovum and the nurse cells increase greatly in size.

Frequently in *Anophelines* a large portion or the whole of the adult ovum consists of a mass of Sporozoa. These consist of numerous small cysts, each containing eight round or crescent-shaped bodies, each with a central chromatin spot (Fig. 48).

The ovarian tube arises in the centre of the ovary, and receives on all sides the follicular tubes. It is lined with a single layer of small cubical epithelium. After passing out of the ovary, a considerable number of striated muscular fibres are arranged in a loose network around it, and pass from it to surrounding structures.

The spermatheca consists of a chitinous sac, with large cells lying externally. These resemble the cells of the cuticle, and contain droplets. They do not

cover the whole of the surface of the spermatheca. The spermatozoa have a narrow, slightly-curved head and a long tail. The duct of the spermatheca is narrow and thick-walled, and contains muscular fibres. Certain large cells lie in connexion with the duct externally. The mucus gland contains cells filled with secretion.

Chapter XII

TO COLLECT AND PRESERVE MOSQUITOES

HOW TO COLLECT MOSQUITOES

Mosquitoes may be collected in two ways :—

1. By capturing the adult flies.
 2. By breeding out from larvae and nymphae.
1. Search in the daytime in houses, huts, and out-houses, at the base of large trees, amidst brushwood and other dark or shaded places. *Anophelines*, however, are rarely caught except in huts and out-houses. They are especially fond of cow-sheds and the darker portions of the eaves of huts.

Some species of mosquitoes may be caught by sitting with a light near a white wall or suspended sheet, or inside a tent, near a jungle or marsh. *Culex* and *Taeniorhynchus* may be found sitting on the surface just beyond the brightly illuminated area. *Anophelines* are rarely caught in this way, but one species at least (*My. barbirostris*) appears to be attracted by light, and was caught by us on an illuminated sheet at night, near swampy land.

In searching for adult *Anophelines*, as many places as possible should be examined, as the distribution of some species is very local.

If the captured insects appear to have fully matured ovaries, some of these should be placed in

bottles, as previously described (p. 91), and allowed to lay their eggs.

If care is taken to place only one species in a bottle, the characters of the ovum may be noted, in addition to the adult insect.

Some of the ova should be placed in fresh water, and an attempt made to determine the characters of the larva when it has hatched out and is sufficiently grown.

2. *Breeding out*.—Full-grown larvae, and especially nymphae, are collected. These are collected from every possible source. Scarcely any water in the tropics will be found free from some form of mosquito larvae. Even strongly brackish waters, containing over one per cent. of salt, often contain large numbers.

Examine water from the following sources :—

(i) Domestic utensils, cisterns, tins, pots, calabashes, boats, etc., in which there has been water for three or four days. The larvae of *Stegomyia*, *Culex*, etc., and only rarely *Anophelines*, will be found.

(ii) Cess pits, pools full of decaying leaves, etc., sewage ditches. Note larvae of certain species of *Culex*, etc.

(iii) Observe presence of the larvae of *Stegomyia* and *Culex* in the water which collects in the axils of banana leaves and other plants. Also, occasionally, *Anophelines* in large collections of water of this kind.

(iv) Puddles of all kinds, with and without algae, ponds, tanks, swamps, rice fields, ditches, canals, rivers, streams, lake margins, and wells, and observe that in all, *Anopheline*, as well as *Culicine* larvae, may abound.

Note that in waters covered with certain species of *Lemna* (duck-weed), *Anophelines* are rarely found.

(v) Examine the larvae (p. 190) and roughly

divide them into as many groups as possible, observing the main characters of each.

(vi) Place each variety in small bottles, over the neck of which a piece of mosquito netting must be tied as soon as the larvae have turned into nymphae.

When the adult insect has hatched out, note its attitude and any other special features.

TO KILL MOSQUITOES

1. A mosquito that has just hatched out from the nympha should not be killed for some hours until its exoskeleton has hardened. If it is killed immediately, the wings on drying will shrivel, and possibly the whole insect become distorted.

2. Allow the insect or insects to escape into a clean, dry, glass vessel.

Pour some chloroform on a pellet of wool and place under the jar, but take care that the mosquitoes do not get wetted by the chloroform. Leave them exposed to the vapour of chloroform some little time after apparent death. Tobacco smoke may also be used for killing, or simply concussion.

After killing, turn the mosquitoes out upon a sheet of clean paper.

TO MOUNT MOSQUITOES*

Necessary apparatus—

Fine silver pins. No. 20.

Thin cardboard or thick paper.

Large entomological or ordinary pins.

Entomological forceps.

Specimen tubes with corks.

* NOTE.—If from any cause it is impossible to mount mosquitoes they may be preserved in the following way:—Without pinning, place in a tube, separating one from the other by tightly-fitting plugs of paper so as to prevent any shaking. The mosquitoes can afterwards be relaxed with benzine. (Newstead.)

1. Prepare a disc by cutting with scissors a circular piece of Bristol board (or very thick paper). The diameter should be slightly less than that of the specimen tube.

2. Push a fine 'silver' pin two-thirds of its length through the centre of this.

3. Place the mosquito upon its back on a clean sheet of paper. (In this and other manipulations use a pin for moving or steadying the mosquito).

4. Take the head of the fine pin in the finger and thumb, or hold it near the head end with a pair of forceps. Endeavour to place the point of the pin exactly in the centre of the origin of the legs, which all arise very close together from the under surface of the thorax. Bear in mind, that the more the insect is touched the more scales are rubbed off, and that a crookedly mounted specimen is better than a 'rubbed' one.

5. Push the pin steadily through the thorax, so that it emerges as near the centre of the dorsum of the thorax as possible.

6. Having transfixed the mosquito, force the point of the pin one millimetre beyond the back, by pressing it against the smooth surface of a cork or tissue paper. The pin should not be pushed through too far, as it prevents the lens of the microscope being brought near enough for examination.

7. Placing the disc against a cork, pass carefully through the edge a stout pin. This is passed in the reverse direction to the fine pin. Force three-quarters of the length of the large pin through the cardboard disc, and then firmly press the point into the cork of a specimen tube, so that when the tube is corked the mosquito is inside (Fig. 49).

It is wise to fix inside the tube a pledget of wool

or some blotting paper soaked in pure creosote in order to prevent mould. Collecting boxes should be moistened to the extent of making the cork shew through the paper (Newstead).

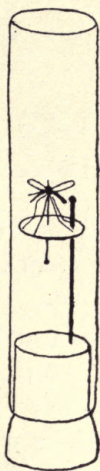


Fig. 49. Authors' Method of Preserving Mosquitoes

Endeavour always to collect both male and female, at least two of each, and, what is of the greatest possible importance for the advance of our knowledge of mosquito classification, the careful description of ova and larvae.

Chapter XIII

ANOPHELINEAE. EXTERNAL ANATOMY OF THE IMAGO

The Head.—Is composed mainly of the two large compound eyes. These meet below and approach one another very closely above.

The following are the usual names for the different regions of the head (Fig. 50)

1. The nape : the extreme back of the head.
2. The occiput : the portion behind the eyes.
3. The vertex : the space between the eyes.
4. The frons : the space in front of the eyes.
5. The gena : the side of the head below the eyes.

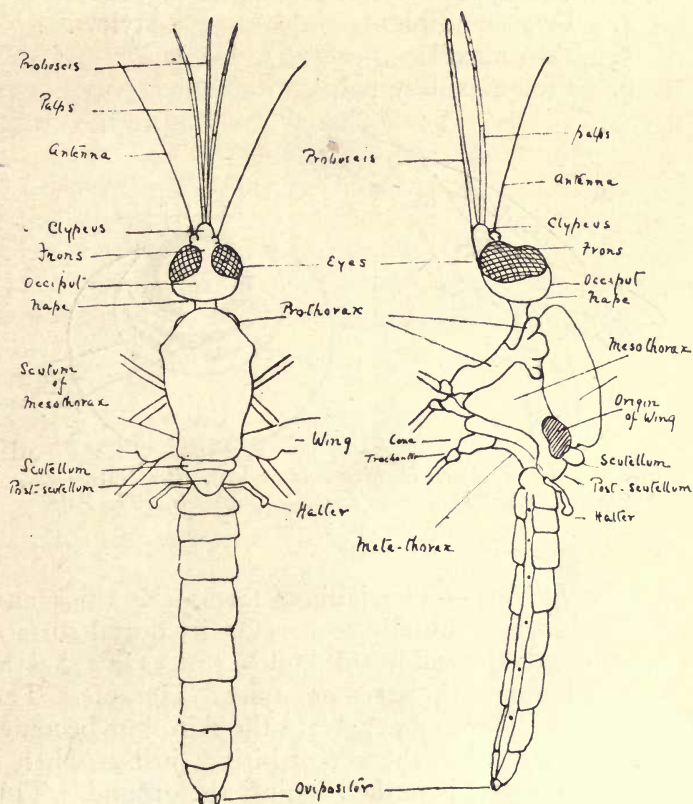
The frons is triangular in shape, with one angle directed downwards. From the upper two angles arise the antennae, and from the lower projects the clypeus, lying over the base of the proboscis.

The Clypeus.—Projects over the base of the proboscis as a prolongation of the frons. The character of the clypeus is of generic importance, *e.g.*,

1. Hairy in *Culex* ;
2. Scaly in *Stegomyia* ;
3. Nude in *Joblotia*.

The Antennae.—Consists of fourteen to sixteen

segments, of which the basal one is large and globular. The plumose antennae of the male readily distinguish it from the female.



50. External Anatomy of Female Anopheline

The Proboscis.—The proboscis consists of the very highly specialized mouth parts, ensheathed in the lower lip or labium. The proboscis consists of (Fig. 51) :—

1. The labium, forming the sheath.
 2. The labrum and epipharynx
or upper lip
 3. The hypopharynx, or tongue
 4. Two mandibles
 5. Two maxillae
 6. Two maxillary palps.
- } forming
the
stylets.

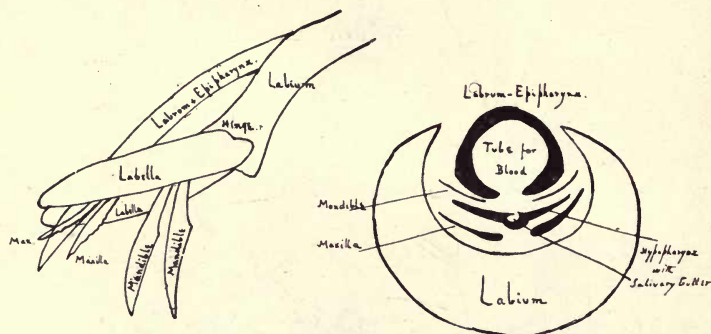


Fig. 51. *The Proboscis (Labium and Stylets)*, after Nuttall and Shipley. Right hand, cross section of Proboscis. The Palpi are not shown

The Labium.—The labium forms the thick and scaly proboscis as usually seen. On its dorsal surface it is hollowed out, and in this hollow run, as in a sheath, the piercing mouth parts or stylets (Fig. 51). The labium itself does not penetrate the skin, but becomes sharply bent during the act of biting, just as when a cane walking stick is pushed against the ground. This may easily be seen if a mosquito is watched during the process of biting.

The Labella.—Attached to the end of the labium by a hinge joint on either side are two leaf-like processes, the labella (Fig. 51). It is through the angle made by the two labella that the stylets pass, as a billiard cue

between the first and second fingers (Nuttall and Shipley).

The labium proper stops short at the point of junction of the labella, but is continued on its upper surface as a blunt point covered with fine hairs (Dutton). We may liken it to a pen continued on beyond the penholder, the junction of pen and penholder being the point at which the labella are hinged on.

Dutton's Membrane.—The area between the end of the labium proper and the extreme tip is covered by an extremely thin membrane (Dutton). In the act of biting, when the labella are separated, this membrane is somewhat stretched, and applied to the skin.

THE ESCAPE OF THE FILARIAL EMBRYO

It has been shown by Low and James that the filarial embryo occurred in the proboscis; according to Low among the stylets. According to Dutton, the embryo really lies in the tissue of the fleshy labium, moreover with its head at the level of the membrane described above, and that it is by the rupture of this excessively thin membrane that the embryo escapes. Grassi and Noe think that the embryo escapes through the middle of the bent-up labium through a rupture at this point, but Dutton's explanation seems more likely.

The epipharynx.—Is the central tube through which the blood is sucked. Its point slopes off somewhat like the tip of a hypodermic needle. In cross section it has the shape of an Ω , the completion of the tube being formed by the apposition below of the hypo-

pharynx. The labrum is blended with the epipharynx, but does not extend to the tip.

The hypopharynx.—Is a thin, flat two-edged lamella closely applied to the under surface of the epipharynx. It is pierced by the salivary duct down which the salivary secretion and sporozoits pass. The opening of the duct is continued as a groove reaching almost to the tip of the hypopharynx.

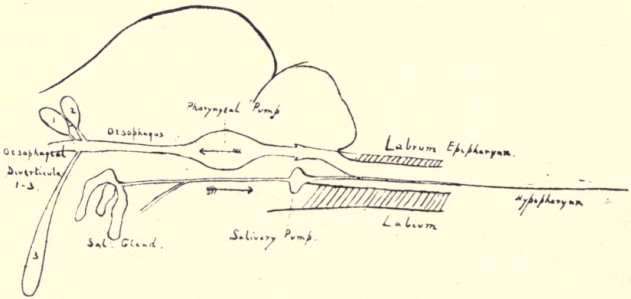


Fig. 52. Showing relation of Pharyngeal and Salivary Pumps to the Proboscis

The mandibles.—Are very fine chitinous rods, in cross section crescentic in shape. At the tip of the mandibles are about thirty serrations, though in certain species of *Culex* these appear to be absent.

The mandibles are closely applied to the sides of the epipharynx.

The maxillae.—Are stouter than the mandibles, and fit around the outer side of these and the hypopharynx. They have about twelve serrations at the extremity, coarser than those of the mandibles. In some *Culices*, papillae replace the serrations.

The Maxillary Palps.—These lie upon either side and somewhat dorsally to the proboscis. In the act

of biting they take no part, but are then separated from and lie at right angles to the proboscis.

The expanded ends of the palpi in the male *Anophelines* are even more conspicuous than the plumose antennae.

The Prothorax.—The main portion of the thorax is mesothoracic; anteriorly, however, there is a collar-like piece of chitin, the prothorax. To this are attached two moveable bodies, the patagia.

The Mesothorax (Fig. 50).—The *scutum* of the mesothorax forms the large globular mass of the thorax. Behind the scutum, and just behind the origin of the wings, is a transverse bar of chitin, the *scutellum*. Behind the scutellum is a convex triangular area extending as far as the first abdominal segment, the *post-scutellum* (Fig. 50).

The scutellum and post-scutellum are of importance in classification.

THE WING

The wings shew :—

1. An anterior straight, thick, and strong border or costa.
2. A posterior curved and thin border, carrying a fringe.
3. Nervures, or veins.

The Costa.—In *Anophelines* is generally covered in part with white, and in part with black scales, forming the spotted margin.

The Fringe.—In *Anophelines* has most frequently light and darker portions, the number and position of which are of some specific importance (Fig. 59).

The Nervures.—In classification, the relative positions of the apices of the two forked cells are

frequently used; also the relative positions of the point where the auxiliary vein cuts the costal vein, and the point where the fifth vein cuts the posterior margin. As a rule, the position of this first point is much nearer the base than that of the second point, but in a few instances, *e.g.*, *My. sinensis*, they almost coincide.

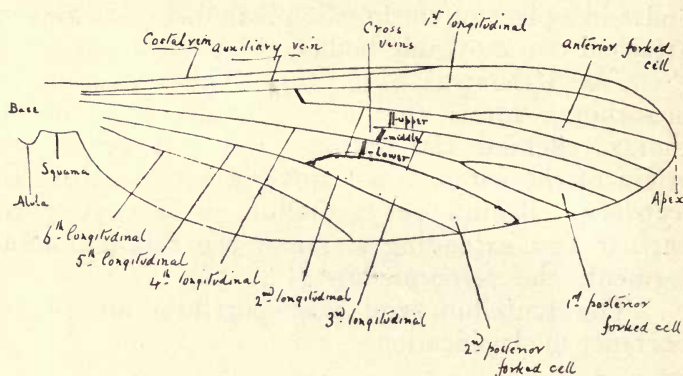


Fig. 53. Wing of *Anophelinae* :—

Upper = Supernumerary cross-vein

Lower = Posterior cross-vein

Anterior forked cell = First forked cell

First posterior forked cell = Second posterior cell

Second posterior forked cell = Anal cell

With regard to the positions of the upper, middle, and cross veins, it will be found that even in the same species there is no constancy, and that they are of little specific importance.

THE LEGS

These consist of the following segments :—

1. Coxa and trochanter: small pieces at the origin of the legs (Fig. 50).

2. Femur.
3. Tibia.
4. Tarsus, consisting of five segments, the last of which carries the claws or ungues.



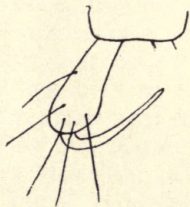
Fig. 54. Fore Ungues of *M. funesta* (♂) the larger Uniserrate.
Fore Ungues of *M. rossi* (♂) the larger Biserrate.
(After Theobald)

The Ungues.—The ungues vary in the male and female and in the different legs. They may be simple, uniserrated or biserrated (rarely triserrated) (Fig. 54). They are of specific value (Theobald).

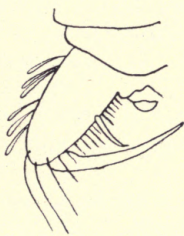
THE ABDOMEN

The abdomen consists of nine segments. To the ninth segment are attached the genitalia.

The genitalia are variously shaped lobed appendages. In the male they are provided at their free end with claspers. The claspers in the male are of specific value (Fig. 55).



A. funestus



A. pseudopiculus

Male Genitalia

Fig. 55. Male Genitalia

Technique.—For studying the chitinous parts, e.g., of the proboscis or genitalia:

1. Boil in ten per cent. potash until sufficiently transparent. If required, bleach the parts by means of chlorine gas (p. 409).

2. Wash thoroughly, stain, if required, with a saturated solution of (basic) fuchsin. Wash.

3. Dehydrate, clear and mount in balsam as for sections.

Chapter XIV

CLASSIFICATION AND IDENTIFICATION OF THE
CULICIDAE.*

The Family Culicidae.—Constitutes a family of flies characterised by possessing :—

- (1) A piercing proboscis about as long as the whole body.
- (2) The presence of scales on the wings characterises the family, the venation of the wings is also characteristic ; scales also occur on the head, thorax and abdomen.

To determine the sub-family.—The family Culicidae is again subdivided into a number of sub-families, based mainly on the length of the palpi in male and female. Theobald divides them as follows :—

TABLE OF SUB-FAMILIES

- A. Scutellum simple, never trilobed. Proboscis straight ; palpi long in ♂ and ♀ - - - ANOPHELINEAE.
- AA. Scutellum trilobed.
 - a. Proboscis strongly re-curved ; first sub-marginal cell very small - - - MEGARHININAE.
 - aa. Proboscis straight ; metanotum nude.
 - β. Wings with six long scaled veins.
 - γ. Antennae with second joint normal in length.
 - δ. First sub-marginal cell as long or longer than posterior cell.
 - ε. Palpi of ♀ shorter than proboscis, of the ♂ long—CULICINAE.
 - εε. Palpi short in ♂ and ♀ - - - AEDINAE.

* In identifying a mosquito, first determine to what *sub-family* it belongs, next to what *genus* in the particular sub-family, and finally to what *species* in the particular genus.

♂♂. First sub-marginal cell very small, smaller than second posterior cell - URANOTAENINAE.

γγ. Second segment of antennae very long—
DEINOCERATINAE.

ββ. Wings with seven long scaled veins—
HEPTAPHLEBOMYINAE.

aaa. Proboscis straight; metanotum with scales or chaetae.

Palpi long in ♂, short in ♀ —
TRICHOPROSOPONINAE.

Palpi short in ♂ and ♀ - DENDROMYINAE.

aaaa. Proboscis elbowed - - - - LIMATINAE.

TO DETERMINE THE GENUS

The scales on the wings and on the body are distinctive of mosquitoes and it is on the character of these scales that Theobald bases his classification into genera. He describes the following kinds of scales (Fig. 56).

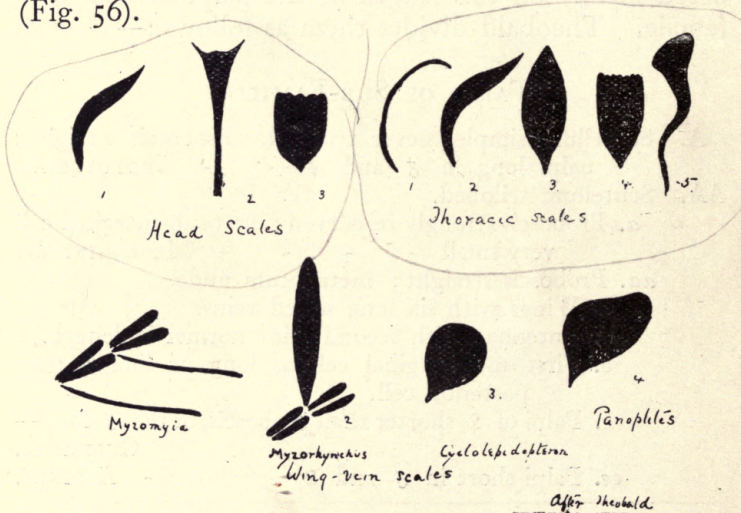


Fig. 56. Varieties of Scales (after Theobald).
Panoplitēs = Mansonia

Head Scales.—(1) Narrow curved; (2) upright forked; (3) flat and occasionally spindle or twisted scales.

In the sub-family Anophelina, examine the genus *Anopheles* (e.g., *A. maculipennis*), note that it has upright forked scales only on the head, and again the genus *Stethomyia* (e.g., *S. nimba*), and note that it has a median patch of flat scales on the head.

In the sub-family Culicina examine the genus *Culex* (e.g., *Culex fatigans*) and the genus *Stegomyia* (e.g., *Stegomyia calopus*, *v. fasciata*). Note that the scales on the head are quite different in the two cases. All mosquitoes belonging to the genus *Culex* have on the head (1) narrow curved and (2) upright forked, but only (3) a few flat scales laterally (Fig. 57); whereas all mosquitoes belonging to the genus *Stegomyia* have on the head (1) no narrow curved scales, (2) a few upright forked, and (3) flat scales covering the whole of the head (Fig. 57).

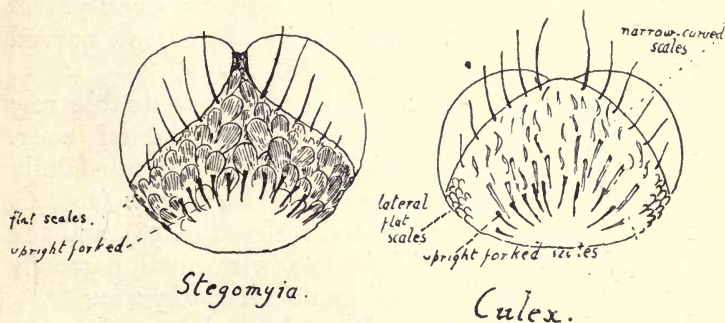


Fig. 57. Head Scales of *Stegomyia* (left) and *Culex* (right)

Although these two mosquitoes are obviously distinct, yet many mosquitoes, which at first sight seem identical, are found to be quite different when their scales are examined.

Thoracic Scales.—(1) Hair-like curved; (2) narrow curved; (3) spindle shaped; (4) flat; (5) twisted (Fig. 56).

In the sub-family Anophelina examine the genus *Myzomyia*, e.g., *M. funesta* or *M. listoni*, and note that the thoracic scales are *hair-like* curved scales; and again the genus *Pyretophorus* (e.g., *P. costalis* or *P. jeyporensis*), and note that the thoracic scales are *narrow curved*.

In the sub-family Culicina examine (a) the genus *Stegomyia* (e.g., *S. calopus*, v. *fasciata*), and note that it has flat scales on the scutellum; (b) the genus *Culex* (e.g., *C. fatigans*), with narrow curved scales on the scutellum; (c) the genus *Mucidus* (e.g., *M. africanus*), with twisted scales on the thorax, giving these mosquitoes their peculiar mouldy appearance.

Abdominal Scales.—In the sub-family Anophelina, the abdomen is generally hairy. Examine the genus *Cellia* (e.g., *C. pharoensis* or *C. pulcherrima*) and note that it has spindle-shaped scales on the abdomen and dense lateral tufts, and that the genus *Pyretophorus* (e.g., *P. costalis* or *P. palestinensis*) has narrow curved scales on the abdomen (Fig. 58).

Wing Scales.—Flat scales occur in a double row along each vein and there are also lateral rows. These scales are most variable, e.g., in the sub-family Anophelina examine the genus *Cyclolepteron* (e.g., *C. grabhamia*), with large inflated scales (Fig. 58), and the genus *Myzomyia* (e.g., *M. funesta*) with small, narrowly lanceolate scales, and the genus *Myzorhynchus* (e.g., *M. paludis*), with dense large lanceolate scales. In the sub-family Culicina examine *Mansonia* (= *Panoplit*es), e.g., *M. uniformis*, or *M. titillans*; the scales have a characteristic broad asymmetrical shape (Fig. 56).

Wing Fringe.—(1) Long narrow pointed scales attached to the edge of the wing by a narrow stalk.

(2) Short scales similar in shape.

(3) Border scales are generally flat but may be variable.

Leg Scales.—Nearly always flat. In the sub-family Anophelina examine the genus *Lophoscelomyia* (e.g., *L. asiatica*), which, as the name implies, has dense scale tufts on the hind femora. In the sub-family Culicina examine *Sabethes* (e.g., *S. remipes*), with its dense tufts (paddles) on the tibia and metatarsus; and *Mucidus* (e.g., *M. africanus*) and *Psorophora* (e.g., *P. ciliata*), with the leg scales elongated and erect.

TO DETERMINE THE SPECIES

Having determined firstly that a mosquito is an Anopheline,* and secondly to what genus it belongs (p. 152) the final step is to find out the species. The most important distinguishing features are:—

(1) *The Costal Spots*.—The main spots on the wing are formed by areas of dark scales on the costal, auxiliary and first longitudinal veins. These spots are fairly constant in each species, but variations occur, e.g., in the typical T spot of *M. rossi* and in the characteristic spot of *N. stephensi* (Fig. 59), and they may not even be the same on the right and left wings, but notwithstanding, the costal spots are of great importance in determining the species.

The Wing Field.—The accumulation of dark scales, here again causes a number of minor spots on the veins, thus the dark areas on the third longitudinal vein in *M. funesta*, *M. listoni* and *M. culicifacies* are

* We only attempt here to describe Anophelines.

different (Fig. 60). Here again variations occur in the same species. An accurate description of a wing should comprise all the minute spots on each vein.

The Wing Fringe.—Where each long vein meets the costal vein (which passes right round the wing) there not uncommonly occurs a light area. Note that the fringes of *M. rhodesiensis*, *M. funesta*, *M. listoni*, *M. culicifacies* differ from one another (Fig. 59).

The Leg Bands.—It must be noted whether the banding, if present, in each segment (tarsus) is apical or basal or both; whether slight or well marked, etc. Thus *N. theobaldi* has the last *two* hind tarsi entirely white, while *N. jamesii* has the last *three* hind tarsi entirely white (Fig. 61).

The Palpal Bands.—The palpi consist of four segments. Accumulation of white scales frequently occur at the junction of two adjoining segments forming a band. Here again variation occurs in the same species especially at different seasons of the year (Adie), so that in this and other markings, *e.g.*, leg bands, species must not be founded on too slight differences.

In *M. rhodesiensis* the tip of the palpi is black, in *M. funesta* white, so these two species are easily distinguished. *P. marshallii* has two broad apical bands and one small basal, while *P. costalis* (otherwise indistinguishable) has one broad apical and two narrow basal, but as all transitions can be found between these two conditions it is doubtful whether they are distinct species (Figs. 60 and 61).

The Male Genitalia.—It is probable that these will give very important aid in distinguishing species, but very few data on this point exist. It will generally be found necessary to stain and mount the genital segments as directed on p. 144.

The Ungues.—Compare those of *M. funesta* and *M. rossi* (Fig. 54).

The Cross Veins.—The position of the supernumerary, mid, and posterior veins is not of much importance in distinguishing species ; as this factor is so variable (Fig. 59).

Larval Characters.—This is an important means of distinguishing species otherwise almost indistinguishable.

N.B.—One precaution must be taken, viz., there must be no doubt that the larva and mosquito in question are of the same species. To make certain of this examine the full-grown larva (p. 191), and then allow the mosquito to hatch out from it, and then examine the mosquito.

Characters of the Ova.—These are also of great help in distinguishing species. Examine the mosquito and then the eggs (p. 187) when they have been laid.

Chapter XV

SUB-FAMILY ANOPHELINA

TABLE OF GENERA*

- A. First sub-marginal cell large.
- I. Antennal segments without dense lateral scale tufts.
- (a) Thorax and abdomen with hair-like curved scales.
No flat scales on head, but upright forked ones.
Basal lobe of ♂ genitalia of one segment.
1. Wing scales large, lanceolate—
Genus ANOPHELES. Meigen.
 2. Wing scales mostly small, long and narrow or slightly lanceolate—
Genus MYZOMYIA. Blanchard.
 3. Wings with patches of large inflated scales—
Genus CYCLOLEPPTERON. Theobald.
Basal lobe of two segments.
 4. Prothoracic lobes with dense outstanding scales - - Genus FELTINELLA, n.g.
Median area of head with some flat scales ;
prothoracic lobes mammillated.
 5. Wing scales lanceolate—
Genus STETHOMYIA. Theobald.
- (b) Thorax with narrow curved scales ; abdomen hairy.
6. Wing scales small and lanceolate ; head with normal forked scales—
Genus PYRETOPHORUS. Blanchard.

* This table is taken from Theobald's *Monograph of the Culicidae of the World*, Vol. IV.

7. Wing scales broad and lanceolate ; head with broad scales, not closely appressed but not forked or fimbriated—

Genus MYZORHYNCHELLA, n.g.

- (c) Thorax with hair-like curved scales and some narrow-curved ones in front ; abdomen with apical lateral scale tufts and scaly venter ; no ventral tuft.

8. Wing scales lanceolate—

Genus ARRIBALZAGIA. Theobald.

- (d) Thorax with hair-like curved scales ; no lateral abdominal tufts ; distinct apical ventral tuft. Palpi densely scaly.

9. Wing with dense large lanceolate scales—

Genus MYZORHYNCHUS. Blanchard.

- (e) Thorax with hair-like curved scales and some narrow-curved lateral ones ; abdomen hairy with dense long hair-like lateral apical scaly tufts.

10. Wing scales short, dense, lanceolate ; fork-cells short - Genus CHRISTYA. Theobald.

- (f) Thorax with very long hair-like curved scales ; abdomen with hairs except last two segments which are scaly. Dense scale tufts to hind femora.

11. Wings with broadish, blunt lanceolate scales—

Genus LOPHOSCELOMYIA. Theobald.

- (g) Thorax and abdomen with scales.

12. Thoracic scales, narrow-curved or spindle-shaped ; abdominal scales as lateral tufts and small dorsal patches of flat scales—

Genus NYSSORHYNCHUS. Blanchard.

13. Abdomen nearly completely scaled with long irregular scales and with lateral tufts—

Genus CELLIA. Theobald.

14. Similar to above, but no lateral scale tufts—

Genus NEOCELLIA, n.g.

15. Abdomen completely scaled with large flat scales as in *Culex*—
Genus ALDRICHIA. Theobald.
16. Thoracic scales hair-like, except a few narrow-curved ones in front; abdominal scales long, broad and irregular—
Genus KERTESZIA. Theobald.
- II. 17. Antennal segments with many dense scale tufts - - Genus CHAGASIA. Cruz.
- AA. 18. First sub-marginal cell, very small—
Genus BIRONELLA. Theobald.

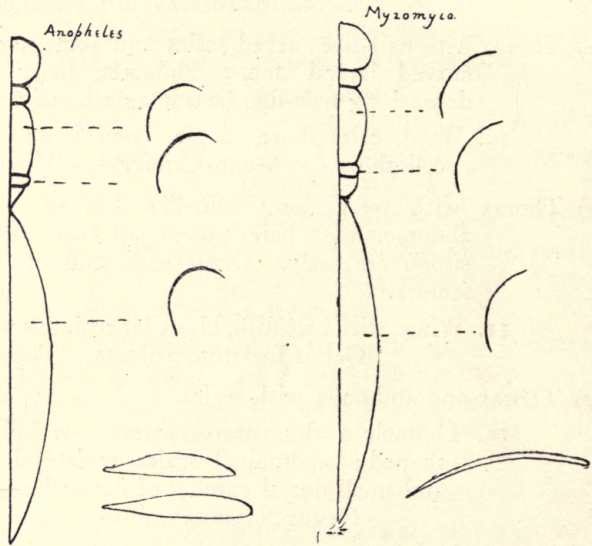


Fig. 58. Thoracic, Scutellar, Abdominal, and Wing Scales of the Anophelinae (after Theobald)

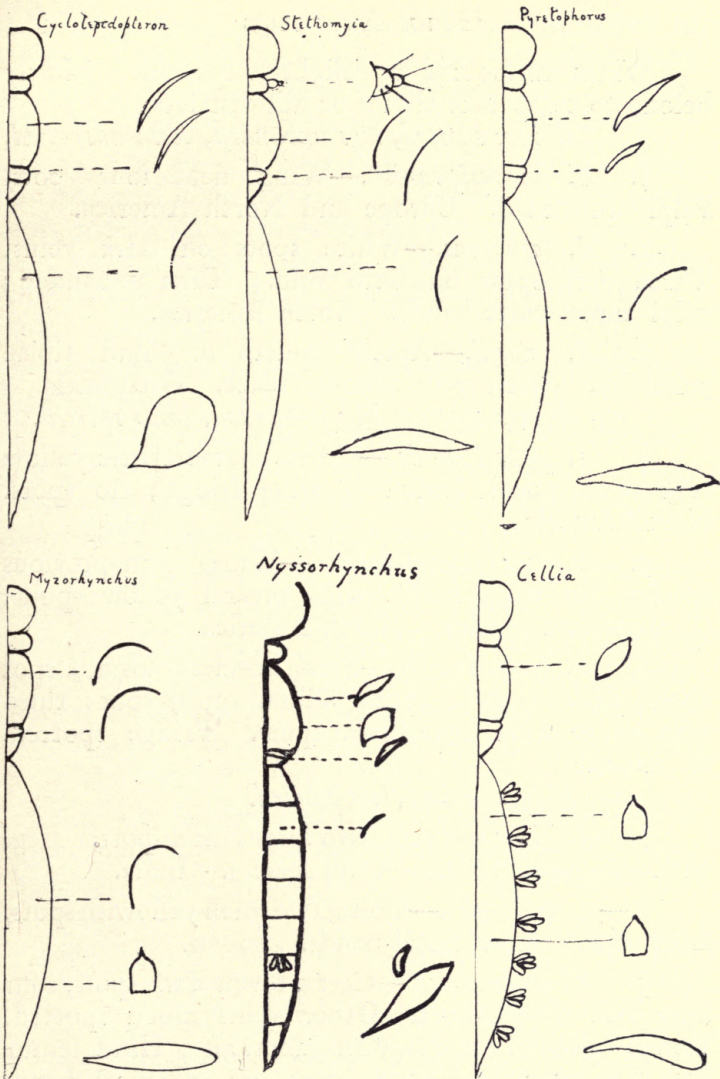


Fig. 58 (contd.)—Thoracic, Scutellar, Abdominal, and Wing Scales of the Anophelinae (after Theobald)

GENUS ANOPHELES

Wings unspotted or slightly spotted. Mostly belong to temperate climes or hill districts.

a. Wings spotted, legs unbanded, costa unspotted.

1. *A. maculipennis**.—Wing field four spots. Palpi, unbanded. Europe and North America.

2. *A. crucians*.—White spots on dark veins. Three dark spots on sixth vein. Tarsi unbanded; palpi three white bands. North America.

3. *A. eiseni*.—Apical fourth of hind tibiae yellowish. Sixth vein wholly black. Guatemala.

aa. Wings spotted, legs unbanded, costa spotted.

4. *A. punctipennis*.—Costa, characteristic yellow spot near apical fourth of wing fringe; no spots. North America.

5. *A. pseudopunctipennis*.—Wings as in previous species but wing fringe with several yellow spots; (?) a distinct species. North America.

6. *A. franciscanus*.—Small species: costa, a spot about middle, and a pure yellow apical spot; third vein white with two black spots. Fringe spotted. N. America.

aaa. Wings spotted, legs banded.

7. *A. gigas*.—Costa, two large costal spots. Legs with pale basal bands. A hill species. India.

8.—*A. wellcomei*.—Costa, two small yellowish spots. Legs with narrow apical bands. Sudan.

9. *A. arabiensis**.—Costa, seven dark spots, four long and three short. Other veins much spotted. Fringe spots at all the vein junctions. Hind femur and tibia speckled—latter often has an apical band.

* Transmits malaria.

Palpi three white bands. Markings vary according to season. Larva: frontal hairs simple. Antennae have no spine. Egg-floats do not meet in middle. Sporozoites found in this species by Patton. Arabia.

10.—*A. dthali*. Costa, four black spots, the basal the longest. First long vein four black spots, other veins pale. Wing fringe, no spots. Palpi, pale with two white bands. Larva: frontal hairs simple. Antennae have a spine. Egg-floats almost meet in middle. Arabia.

aaaa. *Wings unspotted, legs unbanded, thorax with abnormal pattern.*

11. *A. corethroides*.—Resembles *A. bifurcatus*, but differs in (a) thorax being pale brown with a large median anterior dark area, and a long lateral dark area behind this as in *Corethra*. S. Queensland.

12. *A. bifurcatus*.*—Abdomen with golden hairs, thorax with two broad bare lines in front. Europe.

13. *A. algeriensis*.*—Abdomen with brown hairs, lateral scales of veins longer and finer than in *A. bifurcatus*. Anterior and posterior cross veins in same line in both sexes. In *A. bifurcatus* the posterior is internal in ♀, the anterior in ♂. Africa.

14. *A. barberi*.—Differs from previous two in having stalk of first fork cell equal to instead of greater than one-third length of cell. The larva lives in holes in trees. Maryland, U.S.A.

aaaaa. *Wings unspotted, legs unbanded, thorax with normal pattern, second fork cell exceeds half length of the first, palpi banded.*

15. *A. smithi*.—Wing scales very dense. Sierra Leone.

* Transmits malaria.

16. *A. nigripes*.—Not so dense as in previous species. Thorax; grey markings. Europe, America.

aaaaaa. *Wings unspotted, legs unbanded, thorax with normal pattern, second fork cell does not exceed half the length of the first.*

17. *A. aitkeni*.—Bombay Presidency.

aaaaaaa. *Wings unspotted, legs banded.*

18. *A. lindesayi*.—A dark species. Costa: black, apical white spot; hind femora with characteristic broad white band. Hill species chiefly. India.

19. *A. immaculatus*.—An ash grey species. Slight apical bandings to tarsi. Palpi and proboscis lighter at apex. A very rare species. Ennur. Madras.

GENUS MYZOMYIA

To this genus belong those species which are associated in the tropics with the most severe endemic malaria, e.g., *M. funesta* in Africa and *M. listoni* and *M. culicifacies* in India. The group includes, however, several species, one at least of which has, as far as our knowledge extends, no power of transmitting malaria in nature, viz., *M. rossi*.

A. *Proboscis unbanded.*

a. *Legs banded.*

β. *Palpi with four bands.*

1. *M. jehafi*.—Costa, six black spots. Veins much spotted. Palpi, four bands; the apical one may be very small. Egg has no floats and no frill. Aden hinterland.

ββ. *Palpi with three bands.*

Legs with faint basal bands.

2. *M. mangyana*. Palpi, three white bands. Costa, five large spots, two small ones, basal veins much spotted. A very small species. Wing fringe, three spots.

The main costal spot is broken on the first vein as in *M. ludlowii*. Philippine Islands.

3. *M. funesta*.^{*}—Costa: six white spots. Basal spots with pale interruption. Wing fringe: pale spots at ends of all the veins, except sixth. Palpi: three bands, the basal one further from the middle one than the apical. A variable species: third long vein may be dark. Resembles *listoni* and *rhodesiensis* (Figs. 59 and 60).

4. *M. lützi*.^{*}—Characterised by the linear ornamentation on the thorax. Legs with prominent pale apical bands and a broad pale median band to fore and mid metatarsi (first tarsus). Wings, three distinct pale spots; two smaller ones, 3 to 3.5 mm. Rio de Janeiro.

5. *M.*[†] *rossi*.—Probably = *A. vagus* (DÖNITZ). Sumatra. Palpi, somewhat like those of *M. ludlowi*, but easily distinguished; the apical white band is broader; the second band is much nearer the base than in *M. ludlowii*, so that the black area between is longer. Wings, four spots, and some basal spots. The second large spot has the characteristic T shape, but is very variable. Tarsi: slight pale apical and basal bands to some of tarsi. India, Malaysia.

6. *M. longipalpis*.—Palpi long, thin; three narrow white rings; wing costa, black, four almost equal yellow spots; wings mostly brown scaled; hind legs only, banded; narrow apical and basal yellow bands. Length, three mm. Nyassaland.

$\beta\beta\beta$. Palpi with two white bands.

7. *M. aconita*.—*aconita* = unspeckled, because at the commencement of the third long vein the usual dark spot is absent. Palpi, two bands. Costa, four

^{*} Transmits malaria.

[†] Not a *Myzomyia*. Theobald is about to make a new genus for it.

spots, light interruption in basal spot. Fringe, several pale areas. Anterior forked cell much longer and narrower than posterior. Differs from *listoni* in palpi. Sumatra, Java.

γ. Legs banded and spotted.

δ. Upper cross vein straight.

Palpi with three white bands.

8. *M. ludlowi*.—Palpi, apex broad white band; a second small one close to it; a third basal band. Wing costa, four large spots, one or two small basal. Legs, femora, tibiae and metatarsi, especially in hind legs, *spotted* with yellow. Tarsi, broad apical and basal pale banding, especially in hind legs. Length, four to five mm. Philippine Isles.

9. *M. indefinata*.—Third costal spot as in *M. rossi*. Philippine Isles.

10. *M. tessellata*.—Costa, four large, four small spots. Fore tarsi apically and basally banded. Mid and hind tarsi apically only. Thorax, two dark spots in front and a dark area near the scutellum. Malay.

δδ. Upper cross vein markedly curved.

11. *M. pyrethoroides*.—Costa four large and two small dark spots. Wing scales dense. Legs spotted, narrow white apical bands, last hind tarsi dark. Palpi, four white bands. Pretoria.

aa. Legs unbanded.

ε. Palpi banded, apex white.

12. *M. listoni*.^{*}—*Third long vein light*. Wing fringe, four or more light spots (Fig. 59). Palpi, two broad apical bands further apart than in *funesta*, one narrow basal. Basal portion of costa uniformly black (characteristic). Attitude, *Anopheline*-like. Associated with high endemic index in the Duars, Bengal.

* Transmits malaria.

13. *M. leptomerus*.—Base of first long vein white. Anterior forked cell much longer and narrower than posterior. Costa, two spots, thus differing from *hebes*. Fringe, pale areas at all the veins. India.

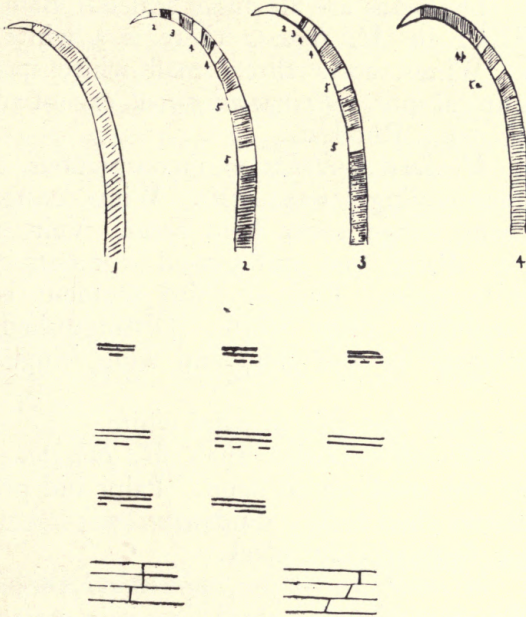


Fig. 59. Wing fringes of (1) *M. rhodesiensis*, (2) *M. funesta*,
(3) *M. listoni*, (4) *M. culicifacies*
Variations in Wing Spots of *M. rossi*, *N. stephensi*, and
P. costalis
Variations of Cross-veins of *M. rossi*

14. *M. culicifacies*.*—Third longitudinal vein dark. Wing fringe, three spots at most. Palpi, three equal bands, two at the joints, one at the apex.

* Transmits malaria.

Attitude, Culex-like. Associated with high endemic index of malaria in the Punjab and Madras.

15. *M. rhodesiensis* (Theo.).—Third longitudinal vein dark. Palps with only two conspicuous bands. The palpi are much longer and thinner than in *M. funesta*. The veins are all dusky scaled. Base of the costa black; in *M. funesta* there is a white interruption. Wings, costa three small white spots and a yellow apical spot. *Fringe unspotted*, except an apical spot (Fig. 59). Rhodesia.

16. *M. hebes*.—*Hebes* = inconspicuous, a small species resembling *rhodesiensis*. Wing costa, four spots; wing fringe, seven light areas. Vein six, one long spot. Palpi, first and second segments covered with white scales. End of third segment is dark, fourth segment quite white. Distinguished from *M. rhodesiensis* by the palpi and wing fringe. East Africa.

ee. Only the apex of palpi white.

17. *M. nili*.—Darker than *M. funesta*. Palpi have only one small apical band. Palpi and proboscis much shorter than body. Sudan.

eee. Apex of palpi black.

18. *M. turkhudi*.—Palpi, *apices black*, the band not so broad as in *hispaniola*; third long vein mostly dark, but varies; pale interruption in basal costal spot. Larvae resemble those of a *Culex*. Ova, very peculiar, type 3 (*vide* p. 188). India.

19. *M. azriki*.—Resembles *M. turkhudi*, but wing fringe no spots. Larva has no branched hair at the end of antenna as in *M. turkhudi*. Position of larva *Culex-like*. Aden hinterland.

20. *M. hispaniola** (Theo.).—Third longitudinal vein mostly pale yellow, except at the base and apex.

* Transmits malaria.

Wing fringe with spots, except where lower branch of fifth and sixth join the costa. Basal portion of costa uniformly black.

AA *Proboscis banded.*

21. *M. albirostris*.—Characterized by the banded proboscis; pale scaled to about half its length. *N. deceptor* has also a banded proboscis. Length, two to five mm. Malaysia.

22. *M. thorntonii*.—Resembles previous but has legs spotted, palpi four bands and proboscis a band near tip. Wing spots resemble those of *P. elegans*. Prothoracic lobes with brown flat scales. Philippine Islands.

ADDENDA

23. *M. bisignata*.—Costa, two white spots, a minute sub-apical one, whereas this is well marked in *M. funesta*. Wing fringe, no spots. Palpi, three bands. Togo.

24. *M. unicolor*.—Palpi, no bands. Costa, three yellowish spots. Togo.

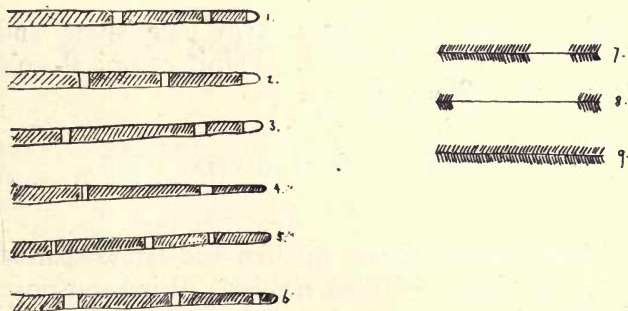


Fig. 60

Palpi of *M. funesta* (1), *M. listoni* (2), *M. culicifacies* (3),
M. rhodesiensis (4), *M. hispaniola* (5), *M. turkbudi* (6),
 Third Long Veins of *M. funesta* (7), *M. listoni* (8),
M. culicifacies (9)

GENUS CYCLOLEPPTERON

Wings with numerous large inflated scales; collected in patches or irregularly disposed (Fig. 58).

Larval Characters (Theobald).—Antenna without lateral branched hair. Clypeal hairs simple. Palmate hairs, six pairs. Lanceolate.

1. *C. grabhamii*.—Palpi unbanded. Jamaica.
2. *C. mediopunctatus*.—Palpi banded, black and gold. Brazil.

GENUS FELTINELLA

Head: upright forked scales with a median tuft of long thin scales projecting forwards. Thorax with hair-like curved scales, and a median tuft of narrow curved scales. Prothoracic lobes with outstanding scales looking like upright forked scales. Abdomen, hairy, except basal segments of male genitalia, which are clothed with flat scales. Basal lobe of genitalia divided into two segments. Wing scales large spindle shaped.

F. pallidopalpa.—Costa, two pale spots and a basal one. Legs unbanded. Palpi ♂, apical golden yellow band. Sierra Leone.

GENUS STETHOMYIA

(Fig. 58)

A. Thorax with a silvery median and lateral bands.

1. *S. nimba*.—A dark species. Wings unspotted. S. America.

AA. Thorax unadorned.

2. *S. fragilis*.—Thorax with long irregular deep brown hairs. Legs long and delicate. Federated Malay States.

3. *S. culiciformis*.—Thorax with a few white hair-like scales. Wings unspotted. Resembles *A. aitkeni*. Has a *Culex*-like attitude. Bombay.

4. *S. pallida*.—Thorax with hair-like brown curved scales and frosty grey down. Philippine Isles.

GENUS PYRETOPHORUS

A. Legs unbanded.

a. Palpi with three pale bands, apex black.

1. *P. nigrifasciatus*.—Costa, four large, two small black spots. Peshin, India, and Cyprus.

aa. Palpi, with three pale bands, apex white.

2. *P. nursei*.—Resembles former. Costa, with four large and two small black spots. Mid-cross vein very long. Branches of second long vein much curved. Quetta, India.

3. *P. minimus*.—Wings, three nearly equal creamy spots and an apical spot. Fringe, spotted except at the sixth vein, thus distinguished from *P. superpictus*. Legs, no trace of banding or pale knee spots. Mid unguis straight; fore unguis curved. Hong Kong.

4. *P. sergenti*.—Costa, with five large black spots, first forked cell much longer than the second. Algeria.

5. *P. palestinensis*.—Wings, five large black costal spots and five yellowish ones of unequal length. Legs brown, a pale spot at junction of tibiae and femora, and tibiae and metatarsi. Palpi, three pale bands, the apex white. Differs from *P. chaudoyei* in the form of the large costal spot in the apical half of the sixth long vein being dark, and in presence of a deep brown median thoracic line. Resembles

closely *P. superpictus*, but the legs are unbanded; spotted wing fringe, and unserrated large fore unguis in the male.

AA. Legs banded.

β . Hind legs only banded apically.

Palpi apex black and three pale bands.

6. *P. myzomyifacies*.—Costa, six black spots. Two small spots on the first vein opposite the large median one. Thorax, three dark lines. Algeria.

7. *P. chaudoyei*.—Wing, six black costal spots; legs banded, a pale knee and tibial spot on the hind legs. Palpi, apex black and with three narrow white bands. Thorax, two dark lines. Algeria.

$\beta\beta$. All legs banded apically.

8. *P. jeyporensis*.—Costa black, two large white spots on the apical half, and two small ones at the base. Fringe spotted. Palpi black, with three white bands, the broadest apical. Apex white. Madras.

9. *P. superpictus*.*—Larva has branched frontal hairs. Wing costa, four distinct spots and additional basal spots. Legs dark brown, with apical white tarsal bands. Palpi, apical white band and two narrower bands, the second is nearer the first than the second is to the third. Europe and Mashonaland.

10. *P. austenii*.—Apical and median palpal bands broad. Costa, two small white spots and a minute third one, spreading on to the first vein. A spot on first vein also, between the two apical costal spots. Spots at base of posterior fork cell, and at apex of lower branch of fifth long vein. Angola.

$\beta\beta$. Fore and hind legs with apical bands.

11. *P. freerae*.—Hind tarsi, three and a half white. Palpi, three bands. Frontal tufts white,

* Transmits malaria.

about as long as head. Costa, seven dark spots. Philippine Isles.

12. *P. cinereus*.—Wings, three white spots on the black costa; wing fringe brown, with yellowish patches. Palpi, four white rings; legs very thin, *jet black*; *apex of femora and tibiae* pure white spot; apices of fore and hind metatarsi, minute apical bands; length five mm. South Africa. B.C.A.

AAA. *Legs spotted and banded.*

γ. Three hind tarsi white.

13. *P. aureosquamiger*.—Wings densely scaled; costa, three white spots. Thorax, golden spindle-shaped scales. Palpi, four white bands. Pretoria.

γγ. Three hind tarsi not all white.

δ. Femora and tibiae spotted.

14. *P. costalis*.^{*}—Wing costa, four large and two small spots. On the first long vein there are two broken spots, under the two middle large spots, giving a pattern only found in *P. marshallii* besides. This arrangement is, however, variable (Fig. 59). Femora and tibiae mottled with yellow. Tarsal banding involves to some extent both sides of the joints. Palpi, three narrow bands at the joints. West Africa, Uganda.

P. costalis v. melas.—Pale costal spots are absent, but the arrangement on the first long vein is the same.

15. *P. marshallii*.—Wing markings very similar to *P. costalis*, distinguished by the palpi; two broad apical bands; one small basal one; apex white. Mashonaland.

Note all transitions between the palpal bandings in these two species.

δδ. Femora, tibiae and first tarsi spotted.

* Transmits malaria.

16. *P. ardensis*.—Tarsal bands, apical. Palpi, four bands. Natal.

ε. A broad tibia-metatarsal joint band.

17. *P. leucosphyrus*.—Leukos = white, sphyron = ankle-joint. Distinguished by prominent tibio-metatarsal band and by the prominent median dark spot on costa. Sumatra.

18. *P. elegans*.—Palpi, four white bands. Wing costa, four large black-scaled areas, three small. Wing fringe, six pale interruptions. Legs speckled with white scales. Femora and tibiae speckled in hind legs. Characterized by a large tibio-metatarsal band on the hind legs. Resembles *N. stephensi*; differs in the palpi; has four, not three, spots on the sixth longitudinal vein. Differs from *P. leucosphyrus* in having four, not six, spots on the sixth vein; in the tarsal banding which is apical only and does not involve both sides of the joint as in *P. leucosphyrus*. India.

ADDENDA

19. *P. atratipes*.—Clypeus, trilobed, costa uniformly black, six spots on the veins. Australia.

20. *P. merus*.—Resembles *P. cinereus*, but distinguished by the spotted and banded femora and tibiae, also by its broader fringe spots. South and East Africa.

21. *P. pitchfordi*.—Three main costal spots, two basal interruptions. Sixth vein two spots. Thorax broad white median band. Zululand.

GENUS MYZORHYNCHELLA

Head clothed with rather long flattened out-standing scales. Not forked and fimbriated. Wings dense, broad short lanceolate scales. Closely allied to *Myzorhynchus*.

1. *M. nigra*. Resembles *P. lützi* but is dark, not fawn coloured, three and a half hind tarsi white. Mid and hind femora white apical spots. Costa, three spots and two basal. Brazil, Mexico.

GENUS ARRIBALZAGIA

Related to *Myzorhynchus*, but has no distinct lateral scale tufts.

1. *A. Maculipes*.—Hind and mid legs much banded and speckled. The 'speckled leg' mosquito, Brazil.

GENUS MYZORHYNCHUS

Palpi densely scaled in the ♀, also the proboscis. These are 'wild' mosquitoes found in situations remote from the dwellings of man. They breed in swamps and large bodies of water, especially those containing weeds. They do not usually frequent houses. *M. sinensis* is, however, attracted by light. They feed readily on human blood when occasion offers (Fig. 58.)

(A) Palpi *unbanded*.—Last hind tarsus brown:

1. *M. barbirostris*, one fringe spot. India, Malaysia.
2. *M. pseudo-barbirostris*.—Distinguished from former by its speckled femora and tibiae. Philippine Isles.
3. *M. bancrofti*, several fringe spots. Australia.
4. *M. umbrosus*, no fringe spot, only one costal spot; wings with light and dark scales. Malaysia.
5. *M. strachani*, no fringe spot, two costal spots, wings mostly dark scaled. Lagos.
Probably identical with *M. obscurus*. Togo.

Last hind tarsus white :

6. *M. albotaeniatus*, other hind tarsi much banded. Malay.

Last two hind tarsi white :

7. *M. coustani*. Madagascar.

(AA) Palpi banded.—Last hind tarsus brown :

8. *M. sinensis*, wing fringe, one pale spot. China.
Palpi banded, last hind tarsus brown, wing fringe unspotted. Apex of palpi white :

9. *M. vanus*, costa two yellow spots, wings distinctly spotted. India, Malay. Philippines, etc.

10. *M. pseudopictus*,* wings without prominent spots. Europe.

11. *M. minutus*, wings, two white costal spots. Punjab.

Apex of palpi black :

12. *M. nigerrimus*. India.

(AAA) Palpi banded, last hind tarsi white :

13. *M. mauritanus*, two hind tarsi white.

14. *M. paludis*, three hind tarsi white. Africa.

15. *M. ziemannii*.—Two and two-thirds hind tarsi white. Africa.

ADDENDUM

16. *Myzorbynchus* (?) *natalensis*.—No ventral scales (? apical tufts). Costa: three white spots, a fourth apical. First long vein: two yellow basal spots, the other spots corresponding to the costal ones except that the first spot has a small additional distal spot. Palpi: apex white, and four other bands. Legs brilliantly spotted and banded. Hind tarsi: $2\frac{1}{2}$ to $2\frac{3}{4}$ white. Broad apical band to first hind tarsus.

*Transmits malaria.

GENUS CHRISTYA

Thorax, hair-like scales and narrow curved laterally. Wings, dense lanceolate scales. Abdomen with characteristic dense long lateral apical tufts of hair-like scales.

1. *Ch. implexa*.—Fore femora with white spots and a prominent pale band. Hind tarsi black, apex of leg white. Uganda.

GENUS LOPHOSCELOMYIA

Resembles *Nyssorhynchus*, but differs in having (1) long curved hair-like scales on thorax instead of narrow curved and spindle scales; (2) dense apical tufts on the hind femora in both sexes.

1. *L. asiatica*.—Very small. Wings, five black spots and four yellow costal spots. Malay.

GENUS NYSSORHYNCHUS

a. Last hind tarsi brown.

Legs spotted.

β. Legs with apical bands.

1. *N. stephensi*.—Syn = *A. metaboles*, Theobald. Tarsus without any segment of hind leg white. Legs brown, speckled with white; joints of fore and hind tarsi with apical spots. Wing costa, four broad prominent black spots and two smaller basal ones. The third largest spot has three typical spots beneath it on the first long vein (Fig. 59). Fringe dark, with pale areas. Palpi, two broad apical white bands, one narrow basal; white scales between the last two bands. India.

2. *N. masteri*. Distinguished from former by the proboscis having pale apex in ♀. It is also smaller. Australia.

ββ. Legs with apical and basal bands.

3. *N. annulipes*. Femora and tibiae banded. Tarsi have apical and basal bands. Palpi: apices of last three segments banded. First and second segments have white scales. Australia.

aa. Last hind tarsi white.

γ. Legs speckled with white.

4. *N. willmori* (James). Wings, four large and three small black areas. Palpi, three white bands, the two terminal ones are equal and broad, the third narrow and basal. Legs, dark brown, thickly speckled with white spots. The last tarsal segment of hind leg pure white. Punjab, Kashmir.

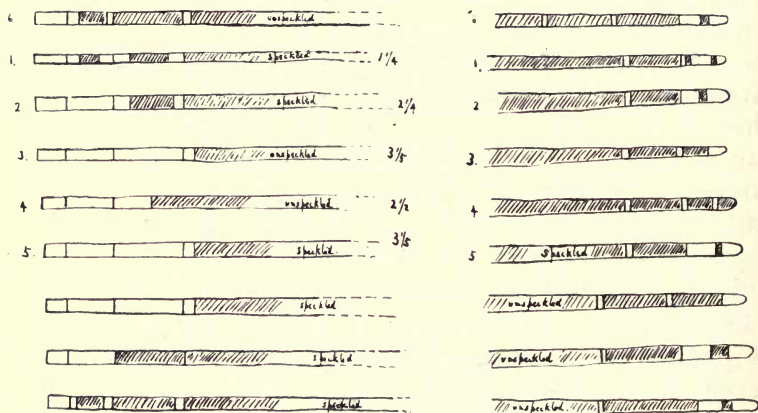


Fig. 61

Hind Tarsi and Palpi of *N. karwari* (6), *N. maculatus* (1),
N. theobaldi (2), *N. fuliginosus* (3), *N. fuliginosus* (nag-
 purensis) (4), *N. maculipalpis* (5), *N. jamesi* (7),
N. pretoriensis (8), *N. willmori* (9)

5. *N. maculatus*.—Resembles *N. stephensi*, but is easily distinguished by tarsi. Wings, costa four large and two small basal spots. Under the third largest spot are three black spots on the first long vein.

Legs, with femora, tibiae, and metatarsi with broken creamy bands and spots. Fore and mid tarsi with narrow yellow bands. Hind tarsi with broad white ones. *Last segment pure white.* Palpi, four bands, two unequal white apical bands, then a small white one, and a second towards the base.

6. *N. bozasi*.—Palpi, three white bands, apex dark. Costa, four chief spots. Femora and tibiae speckled, last hind tarsi white. Fourth tarsus white, with a median black band. Thorax, three black spots, two median, one posterior. N. Africa.

γγ. Legs not speckled.

7. *N. karwari*.—Legs not speckled, one and one-fourth hind tarsal joints white. In fore and mid legs, tarsal joints, except fourth and fifth, have apical white band. In hind legs, first and second tarsus, have apical bands, third and fourth have both apical and basal bands; the fifth is white. Palps, four white bands, two terminal broad and equal, two basal narrow, apex white. India.

δ. Last two hind tarsi white, femora, tibiae and metatarsi speckled.

8. *N. theobaldi*.—Wings, jet black with the costa interrupted by five white spots and an apical spot. Legs, brindled with white scales and a large sub-apical white patch on the femora. Two and a quarter hind tarsi pure white, then a black band, then a small white one. Palpi, three white bands, apex white, two apical bands equal, a third narrow.

A Nagpur variety which Theobald considers may be a distinct species has two-and-a-half hind tarsi white and the tips of the palpi black. India.

9. *N. pretoriensis*.—Clypeal hairs of larva simple. Palps not mottled, otherwise like *N. maculipalpis*. The two white apical bands are further apart. Third

hind tarsus has a small black patch near its base. First tarsus, mottled with white and black, and has a broad white apical band like the second tarsal. The last two hind tarsi only white.

δδ. Last three hind tarsi, white.

ε. Palpi, with three white bands.

ζ. Legs spotted.

10. *N. maculipalpis* = *A. jamesi* in *Reports to Royal Society*, Stephens and Christophers. Wing, costa black with five white spots. Legs, black spotted with white, last three hind tarsi pure white, and apex of next. Palpi, two broad white bands, one apical, a third narrow one towards the base. The rest of the palpi spotted with white. Length, 5.5 mm. India, Africa.

N. maculipalpis, *v. indiensis*.—Hind legs not quite so banded as in the type. Some variation in wing markings.

11. *N. jamesi*.—Costa, four large and two small dark spots. Legs brown, fore femora and tibiae more or less *spotted*. Hind legs, femora and tibiae with an apical white spot, last three tarsi white, and apex of next (Fig. 61). The first tarsal segment of the fore leg has an indistinct median band. Palpi black, with white rings and white apical segment, closely related to *N. fuliginosus*, but easily distinguished. Length, 3 to 3.5 mm. Cp. *A. maculipalpis*, length, 5.5 mm. India.

ξξ. Legs not spotted.

12. *N. fuliginosus*. *—Probably = *leucopus*, Dönitz. Costa, four large and one or more small pale spots. Femora, pale band near the apex. Hind tarsi, three and one-fifth pure white. Palpi, two narrow white bands, apex white (Fig. 53). India.

* Transmits malaria.

13. *N. nivipes*.—Wing resembles that of *N. stephensi*, but legs not speckled. Resembles *N. maculatus* but has three and a fifth hind tarsi white. Malay.

Palpi with four white bands.

14. *N. philippinensis*.—Pale spot at apex of tibiae. Three and a fifth hind tarsi white. Palpi golden brown, three bands.

♂♂. Last two and a half to two and three-quarters hind tarsi white.

15. *N. tibani*.—Resembles *N. theobaldi*. Palpi three white bands sometimes four. Costa, six white spots. First long vein has six spots, the largest consisting of three spots. Aden hinterland.

GENUS CELLIA

Wings densely scaled. Palpi of ♀ densely scaled. Easily recognised by the dense coating of irregular scales.

A. Last hind tarsi white.

1. *C. pulcherrima*, three and three-quarters. Punjab.

2. *C. argyrotarsis** three and a fifth white. W. Indies.

3. *C. pharoensis**, one white, speckled legs.

4. *C. bigotii*, one white, legs not speckled.

5. *C. albimanus** = (*C. albipes*) half white.

AA. Last hind tarsi dark.

6. *C. squamosa*.—A black mosquito with white markings. Three white lateral thoracic lines. Africa.

7. *C. (arnoldi)*, resembles the previous one, but the three white lines are wanting. Transvaal.

8. *C. kochi*.—Pale species. Thorax with two ocelli. Pleurae with large black spots. Malay.

* Transmits malaria.

9. *C. punctulata*.—A large mosquito. Palpi, four white bands. Costa, seven spots. The largest has its component on the first long vein divided into three. Femora and tibiae speckled. Tarsi apically banded. Last two (?) entirely dark. India, Straits Settlements.

ADDENDUM

10. *Cellia jacobii*.—Costa: two basal white dots, three main spots, a fourth apical. Palpi: irregular white bands at apex and last joint; a band at the middle and a few white scales basally. Thorax: three white longitudinal bands and three white lateral bands. Legs, spotted. Tip of last tarsus white in all legs.

GENUS NEOCELLIA

Distinguished from *Cellia* by (1) absence of lateral scale tufts on the abdomen, (2) palpi less scaled in ♀ and (3) smaller wing scales. Theobald classifies the species thus.

a. Last hind tarsi white.

β. Palpi apex black.

1. *N. indica*.—Dehra Dun.

ββ. Palpi apex white.

2. *N. dudgeonii*.—Resembles *N. willmori*, but has not apical and basal banding to tarsi of fore and mid legs, is non-spotted in nature, no white on first tarsi, thorax not dark brown. Head scales black and white, not all white. Kangra valley, India.

aa Last hind tarsi not white.

3. *N. intermedia*.—Femora and tibiae yellow spots, costa six black spots. Palpi apex black. Deesa, India.

GENUS KERTESZIA

Intermediate between *Myzorhynchus* and *Cellia*. Head : upright forked scales. Abdomen : long broad irregular scales. Wing scales as in *Myzorhynchus*.

1. *K. boliviensis*.—Thorax two median brown stripes. Two curved lateral ones. Costa, four yellow spots and a small apical. Veins much scaled. Bolivia.

GENUS ALDRICHIA

Wings much as in *Myzomyia*, for which genus it was originally mistaken.

1. *Al. error*.—Resembles *M. rossi*. Easily distinguished by the abdominal scales. India.

Chapter XVI

THE HABITS OF ANOPHELINES

Seasonal Prevalence.—We have already shewn how selective the *Anophelinae* are in their choice of breeding grounds ; consequently, if at any time, *e.g.*, the dry season, a suitable breeding-ground does not exist, a particular species or genus of the *Anophelinae* may be absent.

Thus we found in Nagpur (India, C.P.), during the dry season, in those places where shallow puddles had dried up, *Mym. rossi* was rare, but it abounded wherever puddles still remained. Where weedy lakes existed, *Nyss. fuliginosus* was common, elsewhere rare. Now these conditions are directly dependent on the rainy season, and where vast areas of weedy swamp are formed during the rains, then *M. nigerrimus* prevailed, to disappear when the swamps dried up. In temperate climes, the temperature is, no doubt, an important factor, the onset of the cold weather causing a general hibernation.

THE HIBERNATION OF ANOPHELINES

1. *Hibernation of the Adult Insects.*—Annett and Dutton describe the finding of *A. maculipennis* during the winter in England in cellars, lumber-rooms, and other cold places, but not in stables where the temperature is higher.

They observed that only females are found, and that these are always fertilized, and have the spermatheca filled with spermatozoa.

The adults of *A. bifurcatus* do not hibernate, or only rarely.

2. *Hibernation of the Larva*.—The larvae of certain *Anophelines*, e.g., *A. bifurcatus*, appear able to resist low temperatures, and are found even when parts of the water are frozen over. Under these circumstances they grow extremely slowly, if at all.

So also in the tropics, different species tide over the 'cold weather' in different ways. Thus James found that *M. culicifacies* hibernated by means of larvae only, little or no growth occurring in these (t. = 55° F. about): whereas *Ce. pulcherrima* and *N. fuliginosus* laid eggs which developed into pupae and imagines.

3. *Hibernation of Eggs*.—There is a certain amount of evidence to shew that eggs can survive for some months in moist earth, exposed to frost, etc. For young larvae have been found in fresh pools in the winter, under conditions that made it unlikely that the eggs had been deposited there on the appearance of water. The resistance of eggs to drying under a tropical sun is, however, practically nil.

MODE OF DISPERSAL OF ANOPHELINEAE

There is no evidence existing at present to show that mosquitoes habitually disperse any considerable distance from their breeding-grounds. In fact, the evidence is completely against such a dispersal, and, broadly speaking, the *Anophelinae* remain where they were developed, and in the native huts where they find abundant food.

That various accidental modes of distribution occur is equally certain, e.g. :—

I. On trains, boats, and even ocean-going steamers, they may be carried long distances, e.g., from West Africa and South America to England, but it

remains to be shewn that *Anophelinae*, thus introduced, ever effect a permanent habitation, even when the removal by this means is from one portion of the tropics to another.

2. Locally, streams and canals may carry larvae and ova long distances, perhaps miles.

3. *Winds*.—The maximum distance that the *Anophelinae* can be carried in this way is quite uncertain. Nearly all of the excessive distances that have been given as possible flights refer to *Culicinae*. It appears certain, moreover, that the *Anophelinae* dislike wind and seek shelter from it.

4. *Trees, Plantations, 'Bush,' Jungle*.—These elements undoubtedly hinder the flight of *Anophelines*, and, on the contrary, open spaces promote their diffusion. It is necessary to bear this fact in mind, where a belt of jungle screens off a source of *Anophelines* (larvae), which may find an opportunity of becoming infected later.

'DOMESTIC' AND 'WILD' ANOPHELINES

Anophelines are mostly found in association with native dwellings where there is abundance of food (blood). *Anophelines* are also generally abundant where cattle are kept.

Certain species are distinctly 'domestic' in their habits, e.g., *Mym. rossi*, *Pyr. costalis*, *Nyss. stephensi*, and others. They are found resting in the daytime in the thatch of huts, and they breed close at hand in the nearest puddle. They may, however, fly up to half a mile if there are no breeding places closer.

Other species are not peculiar to houses, but are also found breeding in streams and pools in the jungle far from habitations. Such species are *Nyss. maculatus*, *Nyss. theobaldi*.

The mosquitoes of the genus *Myzorbynchus*, on the contrary, are 'wild' *Anophelines*. They are only occasionally found in houses. They breed in extensive bodies of water, swamps, rivers, jungle pools, etc. It is *Anophelines* of this type which chiefly frequent one's tent when this is pitched in remote and especially in swampy jungle. The more common species of these wild *Anophelines* are *M. barbirostris*, *M. sinensis*, *M. paludis*.

NATURE OF FOOD

The normal food of the female (domestic) *Anophelines* is blood. In nature they appear to feed every night, the stomach never becoming empty. In *Anophelines* caught under natural conditions, the stomach contents generally shew blood in two or three stages of digestion.

Female *Anophelines* readily drink water, especially if they have been kept for some time in a dry bottle. It seems doubtful whether vegetable juices form an important article of food as appears to be the case with some of the *Culicidae*. Male *Anophelines* can be seen feeding upon banana and other fruit juices, but are, notwithstanding, found dead about the second or third day of captivity.

Bancroft states that *Nyss. annulipes* will live for a month on dates, but only for three days on bananas.

TIME OF FEEDING

The usual time for feeding of *Anophelines* is after dark, more especially in the early night and before dawn. Occasionally some *Anophelines* may be found biting in broad daylight, and Annett and Dutton state that *Anophelines* feed readily in certain parts of

Nigeria by day. Possibly certain species feed more readily by day than others.

We have ourselves seen on rare occasions *M. rossi* attempting to feed in the daytime, and Gray says that *Ce. albipes* when disturbed will bite at any time of the day or night.

On the whole, however, the *Anophelinae* are strictly nocturnal in their habits, nor do they hover around lamps as has been supposed. Of *A. bifurcatus*, Blanchard states that it bites fiercely at dusk, but at night practically not at all. At dawn, however, it begins again, and it bites at all times in shady places, outhouses, etc.

DISTANCE OF FLIGHT

The *maximum* distance that *Anophelines* can fly requires further study. In questions of flight, the species of mosquito should always be noted. In certain villages in India studied by us, *Mym. culicifacies*, *Nyss. stephensi*, and *Nyss. fuliginosus* were always present in abundance, if there were extensive breeding-grounds within quarter of a mile. Where villages were distant half a mile from extensive breeding-grounds, they contained few or no *Anophelines*. The only exceptions to this rule were when breeding-places had only recently dried up. In the case of the above species they undoubtedly fly fairly readily quarter of a mile, but half a mile appears to be beyond the normal distance of flight.

RELATION TO COLOUR, ODOUR OF OBJECTS, ETC.

Anyone who, in the tropics, has left his wardrobe open at sunrise and then closed it, and again examined it some time later, will have often observed the well-known fact that, on his white clothes, few or no

mosquitoes are resting, but that on his blue serge clothes there may be dozens. He will have noted, too, that outside his mosquito net it is on the shady side that the mosquitoes remain longest, until from here also they fly away as the fierce sun rises.

He will have noted, too, that *Anophelines* as well as *Culicines* have a predilection for certain smells. Old boots and blacking attract them strongly, and the leather of a saddle room is their favourite haunt. *Anophelines*, too, much prefer the odoriferous skin of the native to that of the European, as experiments made by us in Sierra Leone clearly shewed.

LENGTH OF LIFE OF MOSQUITOES

The length of life of mosquitoes, under suitable conditions, is probably considerable; several weeks to months.* In captivity they may, if suitably housed and constantly fed, be kept alive for days, weeks, and even months. A mosquito kept some time in captivity becomes infirm, and readily falls into the water whilst laying its eggs. It also finds difficulty in hanging on to smooth glass, and even though a rough surface is supplied the insect is constantly found on the bottom of the cage resting in a horizontal position. After laying eggs, such infirm mosquitoes generally die the same night. In nature, *Anophelines* certainly remain alive in huts for one or two months and possibly longer. After the drying up of all breeding-places, the winged *Anophelines* do not much diminish in number for several weeks. If the drying up continues, the numbers gradually diminish, but specimens may be caught up to two months or more afterwards.

* *Culicines* in England hibernate, *e.g.*, in cellars, from October to April.

‘ AESTIVATION ’ OF MOSQUITOES

In very hot and dry countries, the *Anophelines* which remain through the dry season appear to exhibit some peculiarities in their habits :—

1. Unlike hibernating mosquitoes, they feed regularly and are found full of blood.

2. The ovaries are in the majority large and the ova fully developed.

3. They do not lay their eggs even when ‘ test pools ’ are made near the houses in which they abound.

The Significance of Breeding-Places in the Dry Season.—It is usual in hot and dry climates to find in the dry season at most a few breeding-places. It is a mistake to conclude that these represent the distribution of *Anophelines* in the area in question. Under such conditions careful search will demonstrate that *Anophelines* are present in small numbers in nearly every hut.

THE MALE ANOPHELINE

Little is known as to the habits of the male *Anophelines*. When they are found in numbers it is probably a sign that breeding is going on at the time. At times the number of males caught in native huts exceeds that of the females. Copulation is said only to occur after the first meal of blood. Annett and Dutton describe many hundreds of male *Anophelines* dancing together in midge-like fashion in the villages at dusk.

FECUNDATION

Fecundation, it is thought, takes place generally on the wing. It is also effected in captivity when the *Anophelinae* are confined in test tubes.

Proportion of Males to Females of Reared Insects.

One is often struck by the large proportion of males among mosquitoes artificially raised, a troublesome fact when feeding experiments are being conducted. According to Berkeley, if the larvae are kept supplied with abundant food the proportion of males is much reduced.

ECTOPARASITES OF ANOPHELINES

These are hexapod larvae of Acarines, family Hydrachnidae, the genus possibly *Eylais*, *Hydrodroma*, *Hydrophantes* or *Diplodontus*, but cannot be identified with certainty in the larval stage.

Chapter XVII

ANOPHELINEAE—THE OVUM

THE OVUM

Anophelines in captivity generally lay their eggs on some floating object, but also upon the surface of the water. When laid on a solid object, and even when laid on the water, the eggs are deposited in a piled up mass. Later, the ova, if on water, often form very regular and beautiful patterns. Brick-red masses of eggs are sometimes laid. These do not develop further.

Observe (i) the arrangement in equilateral triangles and star patterns (Fig. 24).

(ii) The arrangement in rows of eggs lying side by side.

Both patterns are dependent upon the shape of the individual ovum ; ova belonging to type 1 forming stars, and ova belonging to type 2, rows.

The number of ova varies, but is usually about one hundred. The size of the ovum varies with different species from about 0.6 to 1.0 mm.

Duration of Egg Stage.—Temperature is no doubt an important factor. Thus the egg stage in *A. maculipennis* lasts from two to four days, whereas in *Ce. argyrotarsis* it is one and a half days in Havana. In *M. rossi* it is about forty-eight hours.

Anopheline ova (with one or two exceptions as yet described) are boat shaped, with an approximately

flat upper surface and a deeply convex lower surface. One end which contains the head of the embryo is blunter and broader than the other. During the act of hatching this end is forced open by the escaping larvae.

1. *The Upper Surface*.—Observe that the upper surface is generally granular or tuberculated in appearance. At either extremity it is continuous with the pointed ends of the ovum, and in this position there are usually several small polygonal areas. The width of the upper surface and the extent to which it is encroached upon by the floats varies in different species.

2. *The Lower Surface*.—The lower surface is generally smooth and dark grey. In damaged ova a silvery membrane will be seen partly detached, shewing a deep shiny-black surface beneath. The silvery membrane is the outer covering of the egg, and formed by the layer of follicular epithelium (Fig. 47). In some species the lower surface is marked with silvery lines forming a reticular pattern.

3. *The Floats*.—Occupying about the middle third of the side of the ovum is a remarkable structure—the float. This consists of a very delicate membrane continuous with the chitinous cuticle covering the whole ovum and containing air cells.

The floats are generally oval in shape and shew regular transverse corrugations. The shape and position of the floats vary considerably in the different species.

4. *The Frill*.—Around the margin of the upper surface (forming the gunwale of the boat) there is in some species a gleaming white frill-like structure. This is striated in appearance, but portions of it may

(in some species) be free from striations. In other species the appearance is rather that of a white striated rim. In all species of *Anopheline* ova yet described, a striated frill or rim is present. The width and extent of the frill vary in different species.

Type 1.—Ova have the upper surface very narrow, with the lateral floats not touching the margin (Fig. 62 : 1).

The species with ova of this type are—

<i>M. barbirostris</i>	<i>M. culicifacies</i>
<i>M. nigerrimus</i>	<i>M. listoni</i>
<i>A. bifurcatus</i>	

Type 2.—Ova having a more or less broad upper surface, with the lateral floats touching the margin (Fig. 62 : 2, 3, 4, and 6).

Species having ova of this type are—

<i>M. rossi</i>	<i>N. fuliginosus</i>
<i>Ce. pulcherrima</i>	<i>N. stephensi</i>
<i>A. maculipennis</i>	<i>A. algeriensis</i>

Type 3.—Ova with no floats, and with upper surface rudimentary (Fig. 62 : 5).

M. turkhudi
M. azriki

Species having ova of the first type have in all cases been species breeding in either open natural waters or running streams.

Species with ova of the second type are in general found breeding in pools.

The only ova as yet systematically described are those of the Indian *Anophelines*. Further observations will probably add further types to the above.

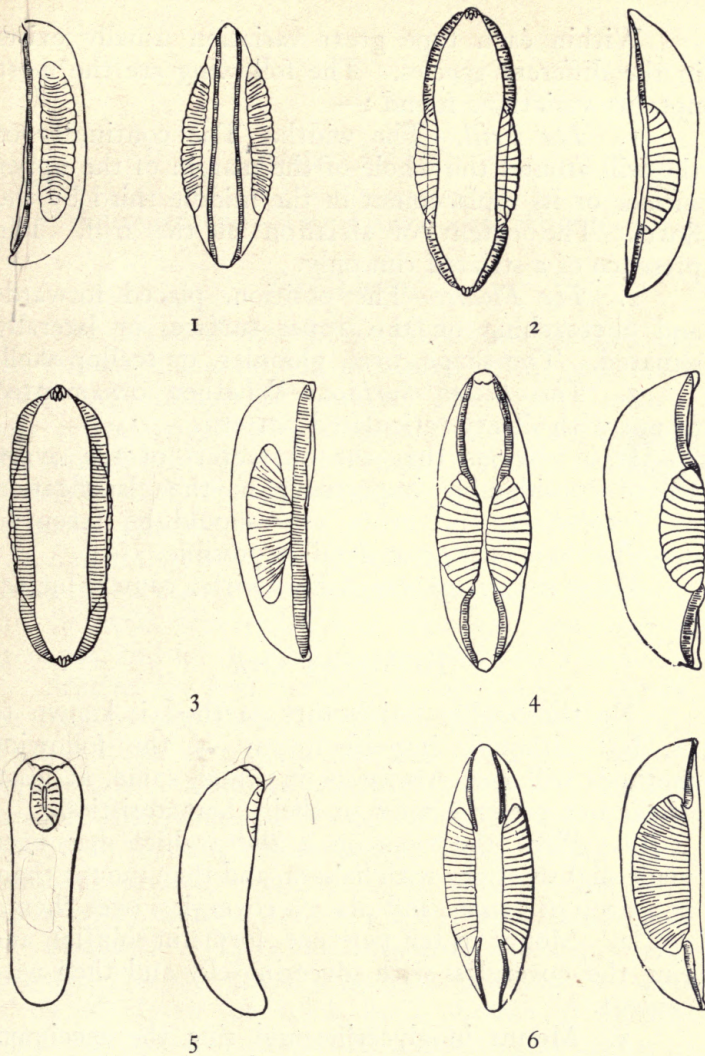


Fig. 62. Ova of *Anophelinae*

- | | | |
|---------------------------|--------------------------|---------------------------|
| 1. <i>M. culicifacies</i> | 2. <i>C. pulcherrima</i> | 3. <i>M. rossi</i> |
| 4. <i>N. stephensi</i> | 5. <i>M. turkbudi</i> | 6. <i>N. maculipalpis</i> |

Within each type great variation usually exists in the different species. The following are the most notable variations found :—

1. *The Frill*.—The width. The continuity of the frill around the whole of the margin of the upper surface or its replacement in the middle third by the floats. The extent of striation of the frill. The presence of a striated rim only.

2. *The Floats*.—The position, placed forwards and encroaching on the upper surface, or laterally situated. The shape, oval, globular, or scallop-shell.

3. *The Lower Surface*.—Whether ornamented or not with silvery reticulated pattern.

It is obvious that the characters of the ovum are of considerable importance in the classification of *Anophelines*, and every care should be taken to describe these in as great detail as possible.

Draw the ova of *Anophelines* with a camera lucida.

TO MOUNT OVA

No thoroughly satisfactory method is known to us, but although imperfect, any of the following methods will give specimens in which some, at least, of the ova preserve most of their characteristics.

1. Place the eggs on a slide which has been made slightly sticky with balsam, and then mount them in a drop of balsam and place a coverglass over them.

2. Mount in ten per cent. formalin solution and ring the coverglass with glycerin jelly and then with cement.

3. Mount in glycerine and ring the specimen.

4. Mount in a drop of cedar-wood oil.

Chapter XVIII

ANOPHELINAE—THE LARVA AND NYMPH

THE LARVA

The larvae of *Anophelines* when first hatched out are minute characteristic creatures, with very black heads and transparent bodies. They move with a very active wriggling movement. They can, even at this stage, be distinguished from the larvae of *Gulicines*, especially with the aid of a lens, as they take up a *horizontal* position.

Examination of the Larva.—This may be done either in the fresh state, when also the process of feeding can be well observed, or a mounted specimen is prepared, and for detailed examination this is best.

Method I.—Boil the larva in ten per cent. potash for half to one hour, until it is fairly transparent. Wash out in water all traces of potash. Stain, if required, in a saturated alcoholic solution of fuchsin (basic). Dehydrate in alcohol, clear in oil of cloves, mount in balsam (Newstead). This method gives beautiful results, and should be used if the larva mounted by method II is not sufficiently transparent.

Method II.—Place the larva in about ten per cent. formalin. When dead, dehydrate in alcohol; clear in oil of cloves (or xylol, etc.), mount in balsam. Place two strips of cardboard under the coverglass in order to support it.

Method III.—Cover the larvae in a drop of water, with a coverglass, and examine with one-half

or one-sixth objective: the following points can be readily made out. Observe that old larvae are often almost totally enveloped in *Vorticella* or other Infusoria.

1. *The Head*.—The head is globular in shape, and is for the most part enclosed in a hard and continuous chitinous case. Anteriorly, there are the rather complicated mouth parts. Posteriorly, there is an opening into which the neck is inserted, around this is a pigmented border resembling a collar. There is a gap in this dark border in the middle line posteriorly, and here two diverging bands of chitin form a 'V' on the back of the head. Grouped around this 'V' mark are more or less continuous patches of pigment, which shew differences in their arrangement, to some extent, specific.

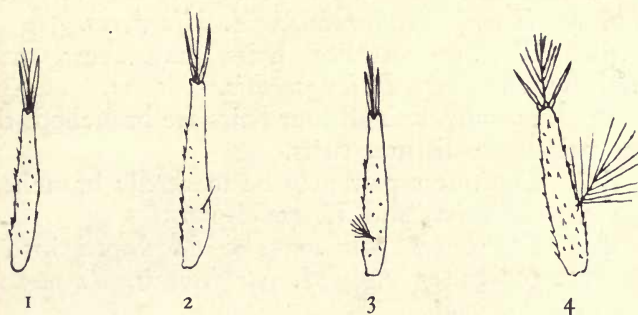
2. *The Antennae*.—Arise from two prominent lateral protrusions, they are freely movable at their articulation. Each antenna is a rod-shaped unjointed body. At its termination are two leaf-shaped bodies, and a branched hair arises between the leaflets. The antenna is covered with small spines, which are particularly developed in pairs along the inner border. In most species of *Anophelines* a hair can be made out arising from a papilla situated at the junction of the proximal and middle third of the antenna.

This hair is of specific importance.

(i) In the majority of *Anophelines* it is simple and unbranched.

(ii) In *A. lindesayi*, *M. nigerrimus*, *M. barbirostris* it is branched, and in the last two very large and conspicuous (Fig. 63).

3. *The Eye*.—In the full-grown larva a crescentic compound eye is seen on either side, and behind this a single pigment mass (simple eye).

4. *The Mouth Parts.*—(Vide p. 80).Fig. 63. *Lateral Hairs of Antennae*1. *M. rossi*2. *N. stephensi*3. *A. lindesayi*4. *M. nigerrimus*

5. *The Clypeal Hairs.*—These are four or six in number (Fig. 64). Two spring from the extreme front of the clypeus near the middle line; two from the outer corner of the clypeus immediately over the feeding-brushes, and two usually very small and not always present behind the origin of the others.

The clypeal hairs are best seen when the feeding brushes are retracted. They must not be confounded with certain other hairs on the larval head. These are :—

(i) Six large branched hairs arising from the prominence lying between the bases of the antennae.

(ii) Four similar branched hairs, but smaller, situated further back (Nuttall and Shipley).

The hairs exhibit great variation in different species, but are quite constant in the one species.*

* Ed. and Et. Sargent describe variations in these hairs in *A. algeriensis*. In eighteen out of forty-six examined both were simple. In three out of forty-six both were slightly branched. In twenty-five out of forty-six the central hair had two or three small terminal branches.

(i) The four anterior hairs may be quite simple and unbranched, *e.g.*, *M. rossi*, *N. stephensi*, *M. culicifacies*, *M. listoni*, *M. turkhudi*, *A. bifurcatus*.

(ii) All four anterior hairs may shew small lateral branches, *e.g.*, *P. jeyporensis*.

In *A. maculipennis* all four hairs are branched, the outer pair form distinct tufts.

(iii) The outer pair may be markedly branched, *e.g.*, *Ce. pulcherrima* and *M. pseudopictus*.

(iv.) The outer pair may be developed into a close tuft (cockade), *e.g.*, *M. barbirostris*, *A. punctipennis*, *Ce. squamosa*.

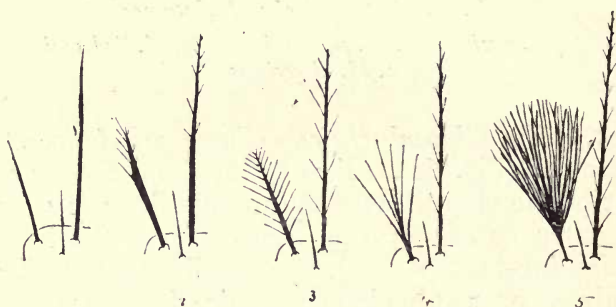


Fig. 64. Clypeal Hairs of Larvae

1. *M. rossi*, *N. stephensi*, *M. culicifacies*, *M. listoni*
2. *N. maculipalpis* 3. *P. jeyporensis* 4. *Ce. pulcherrima*
5. *M. sinensis*, *M. barbirostris*

The two hairs situated behind these may, instead of being very short and inconspicuous, be long and prominent, *e.g.*, *M. turkhudi*.

6. *The Thorax*.—(a) Observe on the dorsum of the thorax a short but extremely stout and strong hair, unlike the others, projecting outwards and forwards.

(b) A flap-like body may, with careful focussing, be seen lying at the base of the most anterior hairs on either side.

(c) In some species of *Anophelines* a single pair of palmate hairs, similar to those on the abdominal segments, are found upon the thorax. In others they are rudimentary or absent. The presence of a well-developed palmate hair on the thorax is of specific importance.

(i) It is well developed and functionally active in *M. culicifacies*, *M. listoni*, *P. jeyporensis*.

(ii) It is rudimentary or absent in other larvae.

7. *The Abdomen*.—The first seven segments are very similar in shape. The eighth carries the opening of the air-tube, and the ninth the anal papillae and large hairs.

8. *The Palmate Hairs*.—The most important appendages of the abdominal segments are certain small fan or palm-leaf-shaped hairs attached by a short stalk to the outer dorsal portions of certain of the segments (Fig. 65).

Place the larva under a coverglass in a drop of water or use a permanently mounted larva.

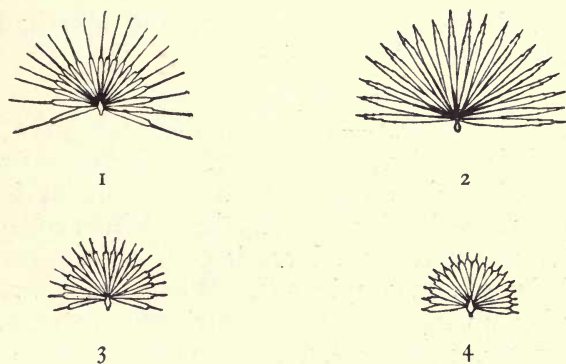


Fig. 65. *Palmate Hairs of Larvae :*

1. *M. rossi*

2. *M. nigerrimus*

3. *M. listoni*

4. *N. maculatus*

Determine the number of segments which carry distinct and large palmate hairs and those carrying ill-developed ones.

1. Fully developed hairs occur on all segments (one to seven) and on the thorax.

P. jeyporensis

M. listoni

M. culicifacies

2. Fully developed hairs on the second to seventh, or third to seventh segments. Rudimentary hairs on the second or even first abdominal segments and on the thorax.

N. stephensi

N. maculatus

N. theobaldi

3. Palmate hairs confined to the third, fourth, fifth, sixth, and seventh segments.

M. sinensis

M. barbirostris

A. maculipennis (Nuttall and Shipley)

4. Palmate hairs confined to the fourth, fifth, and sixth segments.

M. turkhudi

9. *The Leaflets*.—In the well-grown larva each palmate hair consists, as a rule, of nineteen or twenty leaflets arising close together from a short stalk, and forming a semi-circular fan (Fig. 66). When collapsed, as is the case when the larva is beneath the surface, these hairs are inconspicuous. When, however, the larva takes up its characteristic attitude at the surface of the water, these spread out fan-like, and are very striking objects under the microscope.

In the mature larva the leaflets shew much variation in the different species. In most species, the

leaflets terminate rather suddenly in a number of jagged points or notches, whilst the central portion continues as a more or less fine filament.

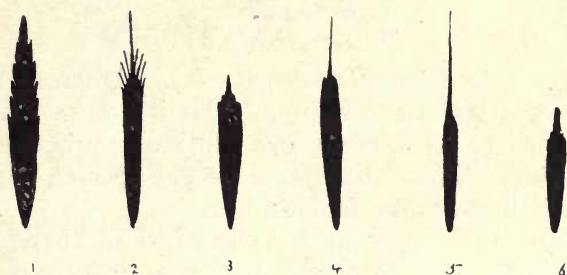


Fig. 66. Leaflets of Palmate Hairs

- | | |
|--|---|
| 1. <i>M. sinensis</i> , <i>M. barbirostris</i> | 2. <i>A. lindesayi</i> |
| 3. <i>N. theobaldi</i> , <i>N. stephensi</i> | 4. <i>M. listoni</i> , <i>M. culicifacies</i> |
| 5. <i>M. rossi</i> | 6. <i>M. turkbudi</i> |

The following types of leaflets are known :—

1. The leaflets are unbrokenly lanceolate in shape, with saw-like notches along the edge of the outer half. There is no distinct terminal filament.

M. sinensis

M. barbirostris

2. The filament is long and filamentous.

M. rossi

M. culicifacies

M. listoni

N. fuliginosus

Further differences are seen in the case of most of the above species. In *M. rossi* the filament is as long as the leaflet, and there is scarcely any notching where the two join. In *N. theobaldi*, the notching is well marked (Fig. 66).

3. The filament is very short, a mere spike-like process.

N. stephensi
N. maculatus
N. theobaldi
N. maculipalpis

10. *The Stigmatic Syphon*.—The eighth segment bears the stigmatic opening. This is a large quadrilateral space, with comb-like chitinous processes on either side. These differ in different species, and are of much importance in classification.

The ninth segment is cylindrical in shape, and is chiefly notable from the fact that it carries four large transparent papillae well supplied with air tubes and certain long curved hairs. Of the hairs, one series projects downwards so as to resemble a rudder. The others project posteriorly. There does not appear to be much variation in the different species.

Duration of Larval Stage.—This is determined by at least two factors. (1) *Food*.—Thus larvae kept in tap-water in the laboratory grow very slowly, if at all. (2) *Temperature*.—Thus the larval stage of *A. maculipennis* varies from sixteen to twenty-two days at air temperatures of 68°-78° F., while in the tropics the time is much shorter, e.g., twelve days for *Ce. Argyrotarsis* in Havana, and eleven days for *M. rossi*, where the temperature of the water varied from 96°-102° F.

PUPATION

Just before this process the larva becomes quieter. The attitude also frequently alters, becoming a hanging one, somewhat like that of a *Culicine* larva.

In this condition larvae are very readily killed by agitating the water (and it is difficult to carry larvae in this stage without killing them).

The change into the nymph is very sudden. A few rapid motions and the larval skin is cast off, leaving the characteristic nymph.

THE NYMPHA

This stage in the tropics usually lasts about forty-eight hours. When first the larval coat is cast the nymph is light in colour, and may be readily overlooked. Later, the nymph becomes darker, and towards the end and immediately prior to the emergence of the imago, *silvery patches* due to collections of air are seen beneath the cuticle.

Pupae taken out of the water and kept on moist blotting-paper will still develop into winged insects (Nuttall and Shipley).

Egg to Imago.—The developmental cycle for *A. maculipennis* is about thirty days at a temperature of 20°-25° C. In the tropics it is much less. Thus the minimum time for *Ce. argyrotarsis*, *M. rossi*, *M. culicifacies* is fourteen days.

Permanent preparations of the nymph are made in the same way as those of the larva (p. 191). Place two strips of cardboard under the coverglass.

Chapter XIX

THE IDENTIFICATION OF ANOPHELINE LARVAE

1. *Naked Eye Characters*.—Some larvae may be identified by the naked eye. The distinction, however, between most species is insufficient to allow of separation by this means.

2. Observe that the colour of larvae is not dependent on species but on the nature of the food, amount of light they have been exposed to in nature, the colour of the water, and other general conditions.

3. The most distinctive of *Anopheline* larvae are those of *M. nigerrimus* and *M. barbirostris*. These are very large larvae, most frequently black, or black, speckled with white, but also brown or vivid green in colour. One of their characteristics is a peculiar 'stick-like' appearance, and the assumption of a bent or contorted attitude.

The larvae of *M. turkhudi* and *M. azriki* can be detected by their attitude, which is almost *Culex*-like. Larvae about to change into nymphae, also frequently adopt this position.

Naked eye examination always requires verification by the microscope.

(A) Larvae may be bred from ova deposited by females of a known species. To successfully accomplish this requires a good deal of care.

1. Remove the paper upon which the ova have been laid (p. 92), and place in a small bottle containing some filtered fresh water from a pool or rain puddle.

2. Place in a good light, but take care that the sun, by the focussing action of the glass, does not heat the water, otherwise the larvae will be killed.

3. When the larvae are hatched, transfer them (after a day or two) to a larger vessel of fresh water containing some weed. When the fresh natural appearance of the water disappears, more fresh water from a pool should be added.

4. By keeping larvae in a not too porous earthenware vessel, they may be placed with impunity all day in the direct sun. It is necessary, however, to watch carefully, to guard against desiccation and consequent death of the larvae.

Larvae kept in flat, partially glazed earthenware vessels, with a certain amount of mud, and placed in the sun, develop more quickly than those kept in bottles.

It is of course necessary to make certain that foreign ova or young larvae are not introduced with the fresh water.

Some larvae are exceedingly difficult to rear artificially, notably those of *M. barbirostris* and *M. nigerrimus*. They remain for long periods without perceptibly increasing in size, and frequently die.

(B) An alternative and less tedious way is to examine nearly-adult larvae found in nature, and to observe, after accurately noting the larval characters, what genera and species of *Anophelines* eventually hatches out.

By examining the larva on a slide with or without a coverglass, the main characters may be noted without in any way damaging the larva, which later becomes a nymph and eventually an imago.

The characteristics of the larvae which are of specific importance are, as we have seen—

1. The antennae.
2. The clypeal hairs.
3. The leaflets of the palmate hairs.
4. The segments carrying palmate hairs.

The following table* will suffice to identify the larvae, so far known, of *Anophelines* (*vide* also ch. xviii).

GENUS	SPECIES	Frontal hairs		External		Antenna shaft		Palmate hairs	
		Branched. Some or all	All plain	Dendriform	Penniform	Branched hair	No branched hair	Filamentous	No definite filaments
Anopheles ...	maculipennis ...	+	—	+	—	+	—	?	?
	bifurcatus ...	—	+	—	—	?	?	?	?
	lindesayi ...	—	+	—	—	+	—	?	?
	aitkeni ...	+	—	—	—	—	+	—	+
Myzomyia ...	rossi ...	—	+	—	—	—	+	+	—
	culicifacies ...	—	+	—	—	—	+	+	—
	elegans ...	—	+	—	—	—	+	—	+
	turkhudi ...	—	+	—	—	—	+	—	+
	funesta ...	+	—	—	—	—	+	+	—
	listoni ...	—	+	—	—	—	+	+	—
Pyretophorus ...	jeyporensis ...	+	—	—	—	—	+	+	—
	superpictus ...	+	—	—	—	—	+	?	?
	cinereus ...	—	+	—	—	—	+	—	+
	ardensis ...	+	—	—	—	—	+	+	—
	costalis ...	+	—	—	—	—	+	+	—
Nyssorhynchus ...	maculipalpis ...	+	—	—	—	—	+	+	—
	maculatus ...	—	+	—	—	—	+	?	?
	fuliginosus... ..	+	—	—	+	—	+	+	—
	theobaldi ...	—	+	—	—	—	+	—	+
	stephensi ...	—	+	—	—	—	+	—	—
	karwari ...	—	+	—	—	—	+	?	?
	annulipes ...	—	+	—	+	—	+	?	?
	pretoriensis ...	—	+	—	—	—	+	+	—
Cellia ...	albipes ...	—	+	—	—	—	+	+	—
	pulcherrima ...	+	—	—	—	—	+	+	—
	squamosa ...	+	—	+†	+†	—	+	+	—
	jacobi ...	+	—	—	—	—	+	+	—
Myzorrhynchus ...	barbirostris ...	+	—	+	—	+	—	—	+
	nigerrimus ...	—	+	+	—	+	—	—	?
	sinensis ...	+	—	+	—	?	—	—	+
	paludis ...	—	+	+	—	+	—	—	+
	natalensis ...	+	—	—	—	—	+	+	—
Stethomyia ...	culiciformis ...	—	+	—	—	—	+	+	—

* This table is based on our own, James's, and Hill's and Haydon's work, and is taken from a paper by the latter authors.

† Either dendriform or penniform.

Chapter XX

THE RELATION OF SPECIES OF ANOPHELINEAE TO MALARIAL ENDEMICITY

Species undoubtedly play an important part in the development of blood parasites in the mosquito.

Proteosoma, for instance, develops in certain species of *Culex*, e.g., *C. nemorosus* was used by Koch in Europe. It does not, however, develop in certain species of *Taeniorhynchus* (S. P. James).

The malaria parasite does not develop in species of *Culex*, *Taeniorhynchus*, *Stegomyia*, or other blood-sucking flies, e.g., *Phlebotomus*, *Simulium*, etc. In the case of *Culex fatigans* placed under absolutely identical conditions with *Anopheles*, no sign of zygote formation occurs on the second or third day.

Similarly with regard to *Filaria*, it is only in certain species of *Culicidae* that certain species of *Filaria* will develop, thus *Ce. argyrotarsis* is an efficient host for *F. bancrofti*, but inefficient for *F. demarquaii*.

The *malarial endemicity* or *endemic index* may be defined as the percentage of infected children (under ten years of age) in any district, and represents the liability of immigrants to contract malaria.

It is well known in a general way that in one country malaria is more intense than in another, but here we have a means of exactly measuring this difference, and, moreover, in the different parts of any particular district. We may illustrate this by the differences we found in Bengal in an extent of country where, as far as we could judge, the climatic conditions were practically identical, yet we find in the environs of Calcutta the endemic index is 0, while in the Duars

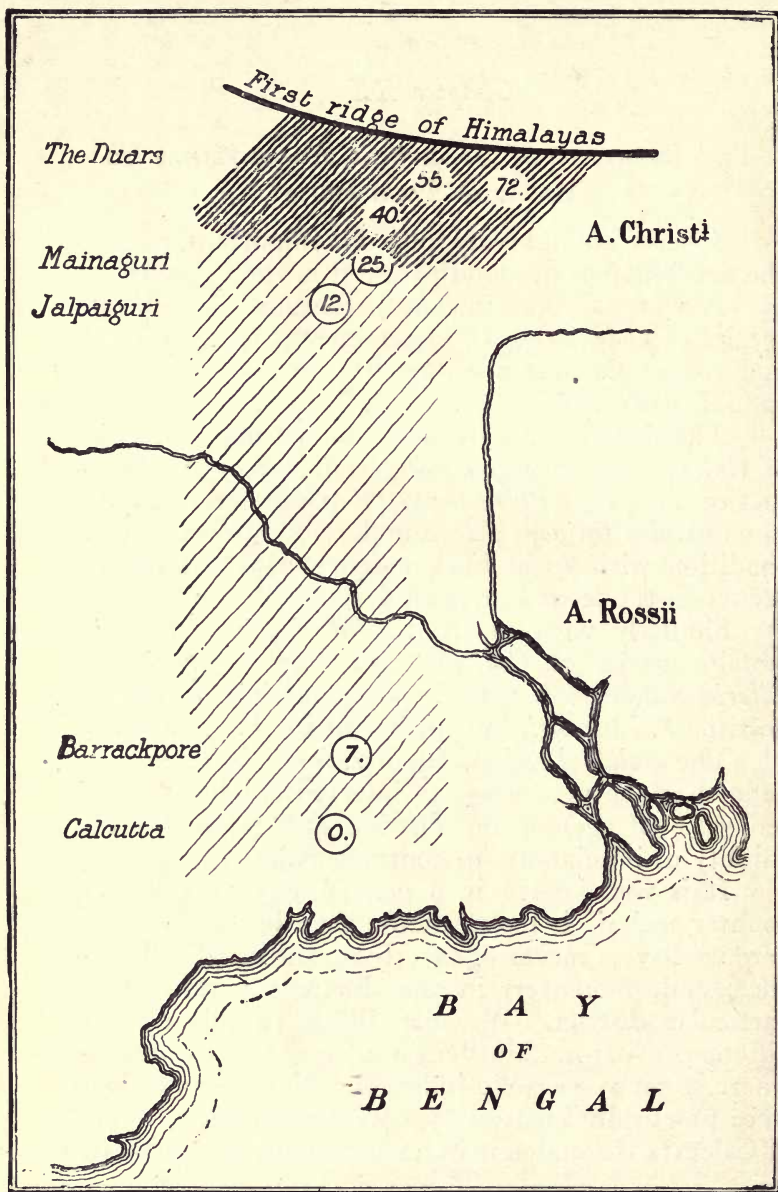


Fig. 67. Showing variations in Malarial Endemicity

(at the foot of the Himalayas) it is as high as seventy-two (Fig. 67). We found, however, that there was one important matter in which the Duars differed from Calcutta, and that was in its *Anopheline* fauna. Whereas in Calcutta *M. rossi* was the predominant species, in the Duars *M. listoni* was the commonest *Anopheline*.

Again, in the Jeypore district (Madras), we had a district of uniformly high endemic index (50-100), and here we found an *Anopheline*, *P. jeyporensis*, which we had not encountered elsewhere, so that the view seemed tenable that the high endemicity of these districts was dependent on their special *Anopheline* fauna. To test to what extent species was concerned in determining endemicity, we then made use of another more exact method, viz., determining by dissection whether any difference occurred amongst the different species in the percentage of infected specimens: we were able to carry this out in the case of *M. rossi* and *M. culicifacies*. We caught these species in the same huts in the same villages at the same time, and determined by actual dissection the percentage of glands infected with sporozoites. The results were most striking, and fully confirmed our previous idea, based on more general considerations of the importance of species. They were as follows:—

I. MIAN MIR (PUNJAB)

		Number dissected	Number with sporozoites	Percentage
<i>M. culicifacies</i>	-	259	12	4.6
<i>M. rossi</i>	- -	496	0	0

II. ENNUR (MADRAS)

		Number dissected	Number with sporozoits	Percentage
M. culicifacies	-	69	6	8.6
M. rossi	- -	364	0	0

Undoubtedly then, under natural conditions, the species is here a very important factor.

Again, under artificial conditions (feeding experiments), we found that there was a difference in the number of zygotes found in the stomach as the result of feeding.

The species which appeared to be most active were :—

M. culicifacies

N. stephensi

N. theobaldi

Those in which zygote formation seemed less abundant were :—

M. rossi

M. turkhudi

M. barbirostris

It should be noted, however, that in these experiments *M. rossi* became infected, while in nature it has never been found infected by us.

There are, moreover, many considerations which lead to the conclusion that in nature all species of *Anopheline* are not equally concerned in the transmission of malaria.

We may have countless numbers of *M. rossi*, as in Calcutta (environs), and yet a malarial index of 0, and this appears to hold good in Madras, Bombay,

and, as far as our observations go, universally. On the other hand, where we find *M. listoni*, *M. culicifacies*, *P. jeyporensis*, in India, we have a high endemic index.

The group of mosquitoes, those associated with intense malaria, are small dark mosquitoes with unbanded legs (*Myzomyia*).

M. funesta AND *P. costalis* IN AFRICA

The former mosquito is, like *M. listoni*, which it closely resembles, a breeder in clean waters, streams, springs, etc., while *P. costalis* is found breeding in shallow pools about houses and frequents towns (in Africa), which *M. funesta* does not.

M. funesta was found by us to be infected in the Lagos hinterland to the extent of twenty-five to fifty per cent., whereas *P. costalis* in Lagos itself was infected only to the extent of three per cent.

ANOPHELINAE THAT ARE KNOWN TO TRANSMIT MALARIA

Although we have about a hundred species, it has been determined, only in a very few cases, which of these actually do transmit malaria in nature. The following list might be extended, as it often is, but only on circumstantial and not on demonstrative evidence.

Europe.—*A. maculipennis*, *A. bifurcatus*, *P. superpictus*, *M. pseudopictus*.

North America.—*A. maculipennis*.

South America.—*Ce. albipes* (W. Indies), *Ce. argyrotarsis* (Brazil), *M. lützi* (Zygotes).

Africa.—*M. funesta*, *P. costalis*, *A. maculipennis*, *A. algeriensis*, *M. hispaniola* (the last three in Algeria); *Ce. pharoensis* (Zygotes).

India.—*M. listoni*, *M. culicifacies*, *N. fuliginosus*.

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Chapter XXI

TO MAKE A MALARIAL SURVEY

ENDEMIC MALARIA

The clue to the epidemiology of malaria in the tropics is to be found in the infection of the native population of a country. The malaria of Europeans is merely the result of their exposure to infection from this source. Investigation into the natural history of malaria, therefore, resolves itself largely into the study of native or endemic malaria. It has always been recognised that in a particular country certain districts are more malarial than others. It was not, however, till Koch used the percentage of infected children as the test of the malarial intensity of a place that accurate measurement of this became possible.

TO INVESTIGATE THE ENDEMIC MALARIA OF A DISTRICT

(A) *The Breeding-Places of Anophelines—*

It is the case in India, and almost certainly will be found to be so in other countries, that certain kinds of breeding-places are preferred by certain species.* A collection of larvae made from shallow puddles will be found to yield quite a different set of species to one made from a streamlet or pool full of vegetation, even though close to the puddles (Fig. 68).

1. Examine all collections of water within half a mile. Stir up the mud of small puddles, and use a dipper where the water is weedy or difficult of access. Examine wells, 'chatties,' streams, and swamps, as

* Thus *M. lützi* is said to breed only in the water collected in the leaves of the parasitic *Bromeliaceae*, and *N. annulipes* is said to breed in the sea.

well as pools of every description. Take specimens of larvae from each, place in specimen tubes and label.

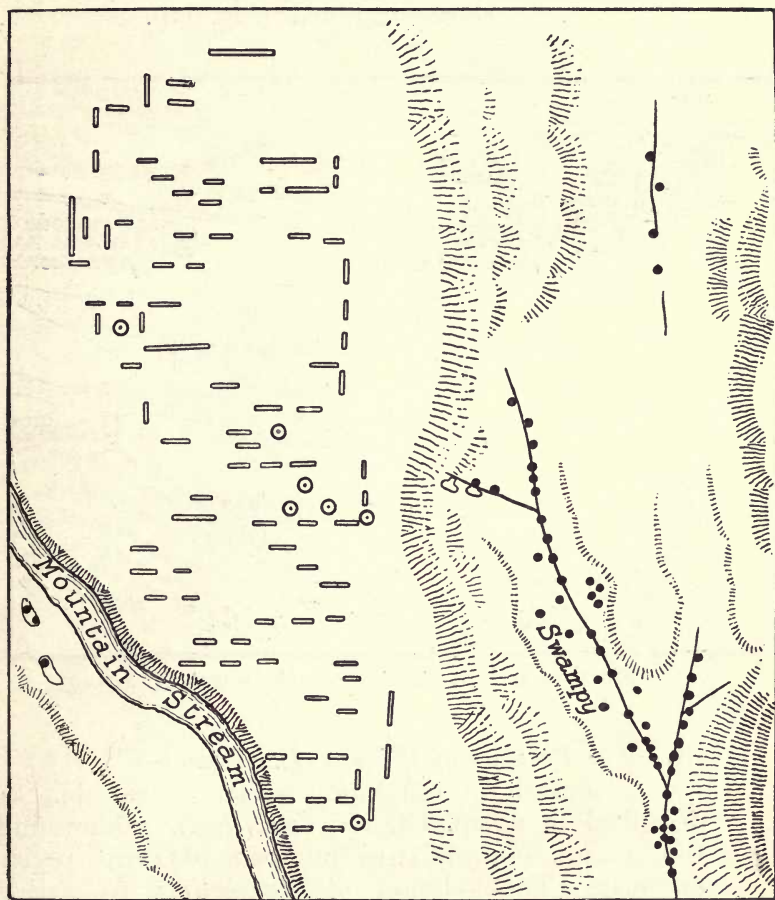


Fig. 68. Portion of Coolie Lines on a Tea Plantation, to show different breeding-places of *M. rossi*, *M. listoni*, and *N. maculatus*

① = *M. rossi*. ● = *M. listoni* and *N. maculatus*

2. Make a map of the neighbourhood, noting—
 - (a) All breeding grounds.
 - (b) What species are found breeding in those examined (Fig. 69).

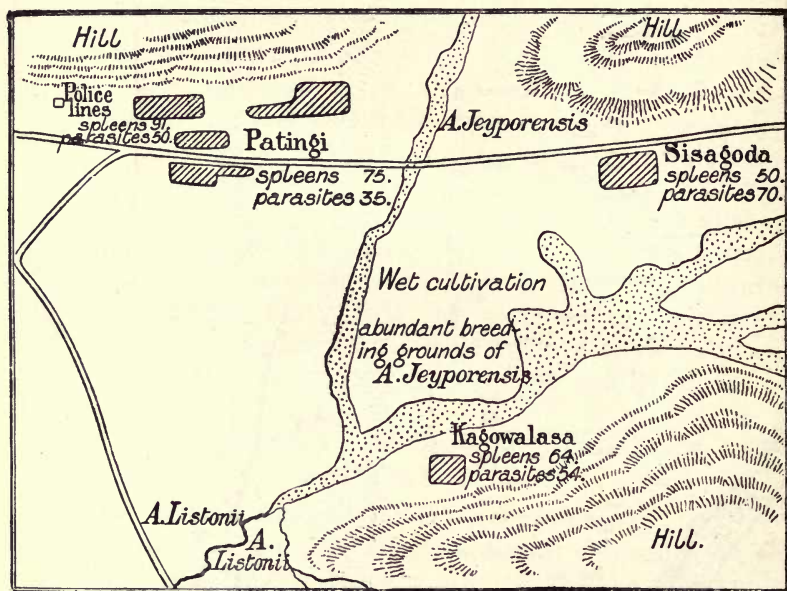


Fig. 69. Map shewing how to make a Malarial Survey

(B) *The Presence of Winged Anophelines—*

1. Search in outhouses, under eaves, etc., as described in Chap. IX, for *Anophelines*. Determine the species, note relative numbers of each species on map. The relation of *Anophelines* to native dwellings will probably be evident.

2. In the dry season the search for *Anophelines* may be negative, and there may be no breeding-places. Make in the most sheltered places small cement pools,

and keep these filled with water. After a certain number of days they may contain young *Anophele* larvae if the adults are present in the houses. (It is necessary to be sure one's water supply does not contain young larvae or eggs). The absence of the larvae in the pools does not necessarily mean, however, that adult *Anophelines* are not present in the houses.

3. In the conditions just described observe the pools made by the first shower of rain of the on-coming 'rains.' Note after three days have passed the presence of larvae in many of these. The distribution of *Anophelines* at the end of the DRY season will usually be found to correspond to that of native huts.

THE PREVALENCE OF MALARIA

If we proceed to ascertain to what extent malaria prevails in a district we may attempt to do so in several ways.

1. We may consult hospital statistics and returns of death from malaria. This method is open to such grave error that it is extremely doubtful whether it is worth the labour bestowed upon it.

2. We may determine to what extent enlargement of the spleen occurs. This method has been largely used.

PRECAUTIONS NECESSARY IN APPLYING THE SPLEEN TEST*

The Age of the Individuals Examined. — The enlargement of the spleen due to ordinary malarial infection tends to disappear once the individual has ceased to suffer from malarial infection. In very malarious countries, after childhood, the

* In countries like India, where kala-azar exists, enlarged spleens from this and other causes may entirely invalidate the 'spleen test' for malaria.

adult population usually shew no splenic enlargement (Tropical Africa).

In less malarious regions a certain number of adults will be found with enlarged spleens and malarial infection. The use then of the percentage of adults with enlarged spleens is not a reliable method of determining the real intensity of malaria.

In the examination of children for splenic enlargement and the presence of parasites in their blood, we found :—

(i) In the early ages, one to two years, the number infected is usually in excess of those shewing splenic enlargement.

(ii) Above two years, the spleen rate is usually somewhat in excess of the parasite rate.

(iii) Above ten years, the spleen rate is usually considerably in excess of the parasite rate.

In the use of a spleen census one should then avoid a mixed adult and child count, and children between two years and ten years of age should be chosen.

By the use of the parasite rate in children up to ten years of age we get a definite and true index of endemicity which may be used in the comparison of one locality with another.

To the last method we would add, as a complementary one, the determination of the percentage of infected *Anophelines* as giving the actual risk of infection in a district.

THE DETERMINATION OF THE ENDEMIC INDEX OF A PLACE

Choose any village or quarter of a town. Get the assistance of a native with local influence, the native magistrate in an Indian bustee, the chief

in an African village. Instruct him to muster the children of the village. The free display of 'pice,' half-pence, etc., will greatly aid one, and by palpating a few spleens previously to taking blood specimens the children will come readily. It is well first to take the blood of one or two adults or big boys so as to allay fears. In all cases it will be found best to take for granted the willingness of the child, and if the operation is quickly and quietly performed there is little objection, especially when each receives payment.

Make twenty to thirty dried films or more.

At the same time a spleen census may with advantage be made.

On examining the films determine :—

(i) Number shewing parasites or pigmented leucocytes in the blood.

(ii) The species of each parasite present and the percentage value for each if the numbers are large enough.

TO DETERMINE THE INFECTION IN THE ANOPHELINES (THE SPOROZOIT RATE)

1. Collect as large a number of *Anophelines* as convenient from the village in and around which the previous observations have been made.

2. Dissect as many specimens as possible, noting in each case the species dissected, and noting in which species, if any, sporozoits are found.

In many cases the sporozoit rate is extraordinarily low, *e.g.*, two per cent., although *Anophelines* are abundant and the malarial index is not low. In others, especially in African bush stations, the percentage may reach fifty per cent.

3. Leave specimens not dissected, for several days, and examine the mid-gut for zygotes.

TO INVESTIGATE EUROPEAN MALARIA

I. Examine the blood of as many Europeans as possible. Enquire *carefully* whether the person is taking quinine at the time, also take the temperature.

- (i) The number shewing parasites or crescents.
- (ii) The presence of pigmented leucocytes.
- (iii) The presence of an increase of the large mononuclear leucocytes.

In every case make a differential count of the leucocytes and keep the record.

Observe especially, any community of Europeans shewing a larger percentage than usual of malarial infection. Note the conditions under which these are living, and note also the probable greater prevalence of blackwater fever in these communities, *e.g.*, Roman Catholic Fathers, West African miners, railway communities, Europeans in poor circumstances living in the slums of native towns, etc., Syrian hawkers, etc. Note those communities habitually taking quinine.

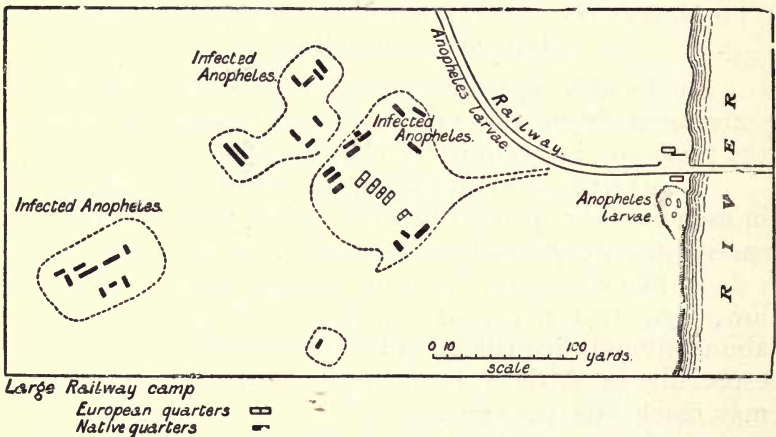


Fig. 70. Shews how Europeans are infected with Malaria from the native (children)

2. Note the usual relation between the degree of ill-health and the proximity of native huts. Make a map shewing European dwellings and shewing huts and hovels in relation with these (Fig. 70).

3. Make a thorough investigation of the conditions in these huts.

(i) The percentage of infected children in each group.

(ii) The degree of infection of the adults.

(iii) Roughly estimate the number of *Anopheles* present, whether swarming, abundant, scanty, or impossible to detect by search. In the latter case make several 'test pools.'

(iv) Determine the species present and the relative numbers of each.

(v) Determine the sporozoite rate for each species.

(vi) Carefully map all breeding-places, noting what larvae are found.

4. Capture as many *Anopheles* as possible in the European houses, especially in the morning, and by looking within the nets. Determine the species, sporozoite rate, and from where probably derived.

In investigating the malaria of any such settlement, native and European, continue the observations if possible throughout the year. Make observations on—

1. Seasonal variations in the endemic index (percentage of infected children).

2. Seasonal variations in the number of *Anopheles* at any time of the year.

4. Distance of flight of *Anopheles* from breeding grounds, etc.

5. Sporozoite rate of *Anopheles* at different times of the year.

6. Examine especially the conditions where *Anophelines*, breeding-places, native huts, opportunity for constant importation of malaria and numerous susceptible children exist, and yet there is a complete absence of endemic malaria. In Africa it will probably be impossible to find such places, but they occur in India.

ENDEMIC AREAS OF A COUNTRY

The map (p. 204) shews how the endemicity of large areas of a country is a very variable one. When opportunity offers, the endemic index should be determined for each locality, and, as far as possible, all the other facts detailed above. But the simple taking of the blood of a number of children (under ten) in any village gives at once valuable information as to malaria of the district, information which often is quite unsuspected. Thus, as is shewn in the map, the endemic index of Calcutta is 0, that is to say, in the immediate environs (not in the town itself) where practically the condition is one of a number of isolated villages, there is no malaria among the native children. At Jalpaiguri the figure is low, twelve per cent., but reaching the foot of the Himalayas, we find the extremely high figure seventy-two per cent. In this case we are able among other differences to find a different species of *Anopheline*, which, as we have seen, is an important factor.

In other cases, however, all the conditions may be apparently identical, but within a distance of even ten miles we may get a change from an endemic index of 0 (Madras) to ninety (Ennur).

These differences hold good in other countries, e.g., in Italy. Here the mortality from malaria in the

north is comparatively trifling, while in the south and the islands it is severe.

Here the difference may be due to differences in climate, but this explanation does not suffice in the examples in India we have mentioned.

Again, we have great irregularities in the distribution of the species of parasite. The quartan, for instance, in the Duars (Bengal) is exceedingly common amongst the native children, but in Lahore it is rare.

Similar differences have been noted in Algeria, where over large areas the quartan parasite is extremely rare, yet in a few localities it occurs in seventy per cent. of cases (Billet).

So in India, as a whole, we have certain small areas where malaria is intense, *e.g.*, the Duars, Jeypore (Madras), and Kanara (Bombay) (Christy), where we also find blackwater fever; yet in others, as in the Central Provinces, where apparently all the conditions are favourable, we have only a moderate intensity.

We require, then, to examine carefully the endemic indices over large areas in order to get an accurate idea of the variations in endemic malaria.

Further, after having established these broad data, it will be necessary to make a close survey of each individual district in order to endeavour to explain the factors at work.

Chapter XXII

CLINICAL STUDY OF MALARIA

THE LEUCOCYTES IN MALARIA

We shall consider (1) the total leucocytes, (2) the percentage value of each kind.

The Total Leucocytes.—We may take 10,000 as the normal value per mm.³, and as 5,000,000 is the normal value for red cells, the proportion of white to red is

$$\frac{WC}{RC} = \frac{10,000}{5,000,000} = \frac{1}{500}$$

Now, in malaria, we may find two conditions, either that the total number of leucocytes is considerably below the normal value, 10,000, *i.e.*, there is leucopenia or hypoleucocytosis, or that the total number is much above 10,000, *i.e.*, leucocytosis. If there is leucopenia, say, for instance, the total number is 5,000 instead of 10,000, then

$$\frac{WC}{RC} = \frac{5,000}{5,000,000} = \frac{1}{1,000}, \text{ i.e., the fraction } \frac{WC}{RC}$$

is smaller than normal.

If, on the contrary, the total leucocytes are 20,000 instead of 10,000, *i.e.*, leucocytosis, then

$$\frac{WC}{RC} = \frac{20,000}{5,000,000} = \frac{1}{250}, \text{ i.e., the fraction } \frac{WC}{RC}$$

is greater than normal.

It is this ratio $\frac{WC}{RC}$ that it is important to determine, for unless the red cells are counted as well as the white, little value attaches to the leucocytic value.

In malaria, we find that we get changes of the following kinds :—

(1) 11 a.m., rigor. Red cells = 2,900,000. $\frac{WC}{RC} = \frac{1}{290}$
 White cells 10,000 - - -
i.e., leucocytosis.

(2) 11.30 a.m., rigor completed - - $\frac{WC}{RC} = \frac{1}{764}$
i.e., leucopenia.

(3) 2 p.m., temperature 38.2° - - $\frac{WC}{RC} = \frac{1}{968}$
i.e., increased leucopenia.

The leucocytosis was, in this case, quite transient, followed by a marked leucopenia.

During the course of an attack, we may have changes of this kind :—

1. Some days before the attack and before parasites appear in the blood, instead of

$$\frac{WC}{RC} = \frac{1}{500}, \quad \frac{WC}{RC} = \frac{1}{1,000} \text{ i.e., a leucopenia.}$$

2. During the shivering attack and height of the pyrexia, the condition changes to one of leucocytosis, so that

$$\frac{WC}{RC} = \frac{1}{300}, \quad \frac{1}{200}, \text{ or even } \frac{1}{90}$$

3. This leucocytosis may not last long, but is

followed again by a marked *leucopenia* which is at its maximum before the onset of the next attack.

$$\frac{WC}{RC} \text{ instead of } \frac{1}{500} \text{ may be } \frac{1}{800}$$

Billet (Fig. 71), who has traced out hourly the relation of the leucocyte curve to the temperature curve, has shewn that in regular curves of the tertian or quartan type, the leucocytic curve follows closely the variations in the temperature. Thus, before the febrile attack in a quartan, there may be a leucopenia represented by $\frac{WC}{RC} = \frac{1}{1200}$; at the time of the attack,

however, there is a leucocytosis of $\frac{WC}{RC} = \frac{1}{200}$.

This gradually disappears, passing through the normal value $\frac{1}{500}$, and again reaching a marked *leucopenia* before the next attack. The variations are of the same kind in irregular temperatures, the leucocytosis corresponding to the rise of temperature, and the leucopenia to the apyretic intervals.

The Percentage Value of the Leucocytes.—If we now make a differential count in a stained specimen we shall be able to ascertain what change, if any, there is in the relative percentage of the different kinds.

1. The main *characteristic* change is that there is an increase in the percentage of large mononuclears, so that at times they may even outnumber the polynuclear.

2. The change is especially well-marked in the periods of apyrexia (*i.e.*, when there is a leucopenia). When there is a leucocytosis the increase in the mononuclears may not be apparent.

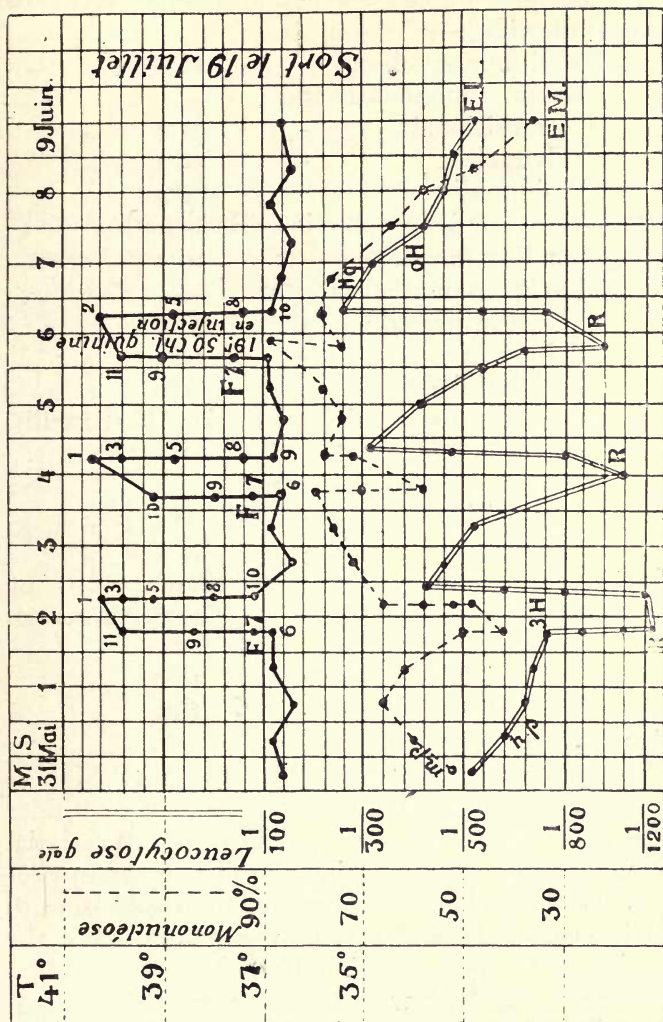


Fig. 71. Showing the changes in the total Leucocytes (and in the percentage of mononuclear large and small) in a case of simple tertian fever. The double line curve = that of total Leucocytes. (After Billet)

As examples of the leucocytic change, we may give the following :—

(i)	Small mononuclear	-	-	18.1 per cent.
	Large mononuclear and			
	transitional	-	-	31.4 „
	Polynuclear	-	-	50.2 „
	Eosinophil	-	-	0.4 „

A fatal case of malignant tertian (Bastianelli).

(ii)	Small mononuclear	-	-	19.1 per cent.
	Large mononuclear and			
	transitional	-	-	41.0 „
	Polynuclear	-	-	39.0 „
	Eosinophil	-	-	0.6 „

A fatal case of comatose malignant tertian (Bastianelli).

(iii)	Small mononuclear	-	-	18.1 per cent.
	Large mononuclear and			
	transitional	-	-	26.4 „
	Polynuclear	-	-	55.3 „

Malignant tertian fever, t. 37.2° C. (Panse).

(iv)	Small mononuclear	-	-	14.8 per cent.
	Large mononuclear and			
	transitional	-	-	46.7 „
	Polynuclear	-	-	38.5 „

Malignant tertian, t. 97.6 F. (S. and C.)

The figures are by no means always as high as this, but, as we have already said (p. 43), we consider a value above fifteen per cent. as diagnostic of malaria. The higher values are appreciated at once by an inspection of the slide where the large mononuclears seem to occur in every field, and may be pigmented. For the low values a careful count is required.

Further, together with the increase of the mononuclears in malaria there are, if thorough search is made, also pigmented leucocytes to be found. The *relative* count of malaria is of great assistance in at least two

conditions, (1) in those cases where quinine has been taken, (2) where consequently the diagnosis is uncertain and the question of typhoid fever, etc., arises.

TRYPANOSOMIASIS

An increase in the large mononuclears has been found in human trypanosomiasis, but it would appear as if an increase in the lymphocytes were the characteristic change in sleeping sickness. The possibility of this should, therefore, be excluded by gland puncture.

TYPHOID FEVER

During the first week (of uncomplicated cases) the leucocytes are normal.

During the second week there is a *leucopenia*, e.g., 2,000, and the leucopenia is in proportion to the severity of the disease.

During the third and fourth weeks the *leucopenia* is still more marked, though also a leucocytosis may be found without any apparent cause.

Relative Leucocyte Values.—During the third, fourth, and fifth weeks the mononuclears, *large and small*, may reach the values of forty to sixty per cent., and among these the proportion of *small* mononuclears is very striking.

PNEUMONIA

There is very early a leucocytosis, e.g., 25,000, four hours after the initial chill. The maximum occurs, as a rule, just before the crisis. The number may fall from a high value to normal in twenty-four hours. Leucocytosis is said to bear a relation to the amount of exudation (*i.e.*, lobes involved).

Relative count—

Large and small mononuclear	2 to 4 per cent.
Polynuclear - - - -	90 to 95 „
Eosinophil - - - -	rare.

THE ISOTONIC POINT OR TONICITY OF THE BLOOD

If a drop of blood is allowed to drop into a one per cent. solution of salt in a small test tube and stirred up, the uniformly turbid solution will eventually become clear when the corpuscles have settled at the bottom and the supernatant fluid will be unchanged ; if, on the contrary, we add another drop of blood to a little water in a test tube the whole drop is immediately *laked*, and we have resulting a solution of haemoglobin. The former solution of salt is called hypertonic, the latter solution of water hypotonic. Now, if we start with such a hypertonic solution, one per cent. salt, and proceed gradually to dilute it, we shall eventually reach a strength where the hypotonic, *i.e.*, haemolysing effect begins to appear. The strength of salt solution just above this where no change occurs is the isotonic point for the particular blood in question. This point then gives us information as to the resistance of the corpuscles to a haemolytic agent. The blood in various diseases is found to vary in regard to the strength of salt required to prevent haemolysis. So that if a normal blood is unchanged by a 0.5 per cent. salt solution, whereas an abnormal requires 0.6 per cent. to protect it, the latter blood is described as having a less resistance than the former, but it has a *higher* isotonic point.

The determination of the isotonic point then gives us a more definite notion of the state of the blood in disease than does a mere determination of

the haemoglobin. The isotonic point of human blood is about 0·41 per cent. salt solution.

TO DETERMINE THE ISOTONIC POINT

1. Measure out one c.c. of each salt solution of descending strengths, 0·43 per cent., 0·41 per cent., 0·39 per cent., etc., into four small test tubes and one c.c. of water into a fifth tube.

2. Add to each the amount of blood (or the blood may be washed free from serum by centrifugalizing and used as an emulsion of known strength in salt solution) contained in two divisions of the stem of a Thoma-Zeiss pipette (the whole stem contains ten divisions).

3. Allow to stand for some time. Some of the solutions will have haemoglobin in solution.

In malaria, the resistance of the blood is lowered, thus, whereas in a control normal blood a 0·41 per cent. salt solution gave no haemolysis; in the case of two malaria patients, the haemolysis was well marked.

In blackwater fever, on the contrary, a raised resistance of the blood may be found.

THE URINE

It is especially in blackwater that we still require complete analyses of the urine, and more especially in those who are constantly subject to malarial attacks and are at the same time taking quinine. It is possible that such analyses might give us indications which would enable us to avert the danger of an attack of blackwater fever and to determine when quinine should not be given.

Albuminuria.—The occurrence of albuminuria in malaria varies according to the particular country;

thus in Rome it is uncommon, in Senegal, on the contrary, exceedingly common. This is an illustration of the often neglected fact that tropical malaria differs in many ways from malaria of temperate climes.

Filter the urine if morphological constituents are present, as is the case in blackwater fever, through two thicknesses of filter paper, or add some calcined magnesia, then filter. Place some urine in a urine glass and, with a pipette reaching to the bottom, allow half as much nitric acid to slowly trickle in (Simon). A white cloud at the junction layer indicates *serum albumin* (globulin or peptones). Urea nitrate crystals will often separate out at this junction layer.

Serum Globulin.—Make the urine alkaline with ammonia; filter off any precipitated phosphates; to the urine add an equal volume of saturated solution of ammonium sulphate. A precipitate indicates globulins; or the formation of the precipitate may be seen at the junction layer. Test filtrate for albumin by adding excess of acetic acid and boiling.

Albumoses.—Acidify the urine with acetic acid; add an equal volume of a saturated solution of salt; boil; if a precipitate occurs (albumen) filter hot. Albumoses separate out on cooling; or to the hot filtrate add caustic soda solution, then dilute copper solution gradually; a red colour signifies albumoses.

NOTE.—Urices rich in urobilin (*e.g.*, malaria and blackwater fever) will give this biuret reaction.

In presence of urobilin: to ten c.c. of urine add eight grammes of powdered ammonium sulphate until dissolved; boil for a few seconds; the albumoses are precipitated on the sides of the test tube; pour off the urine, and wash the precipitate with alcohol, then chloroform; dissolve in water and apply the biuret test. Test the alcoholic extract for urobilin.

Nucleo-Albumens.—Filter the urine carefully; boil to remove albumen; then add gradually excess of strong acetic acid. A turbidity indicates nucleo-albumens.

BLOOD (HAEMOGLOBIN, ETC.)

1. *Examine Spectroscopically* (Fig. 72). — If the bands of methaemoglobin or oxyhaemoglobin are seen, confirm by adding ammonium sulphide when the bands of reduced haemoglobin are got.

2. *Heller's Test*.—Make the urine strongly alkaline with caustic soda; boil; the precipitate in the presence of haemoglobin is bright red; confirm by dissolving the filtered precipitate in acetic acid, a red solution is formed (spectroscopically this gives the characteristic bands of haemachromogen).

3. *Guaiacum Test*.—Equal parts of tincture of guaiacum and oil of turpentine (which has been exposed to the air) are taken; add slowly to the urine. A blue ring is formed at the junction layer. (Unreliable).

METHAEMOGLOBIN

The urine in blackwater fever when examined early, most frequently contains blood pigment in this form, later oxyhaemoglobin. This, according to Hoppe-Seyler, also holds good for every urine with haemoglobin in solution.

The Characters of Methaemoglobin are :—

In *acid* solution the oxyhaemoglobin bands are weak or invisible. There is a band *between* C and D, nearer the former. The band of acid haematin is similar in position. It is, however, close to C.

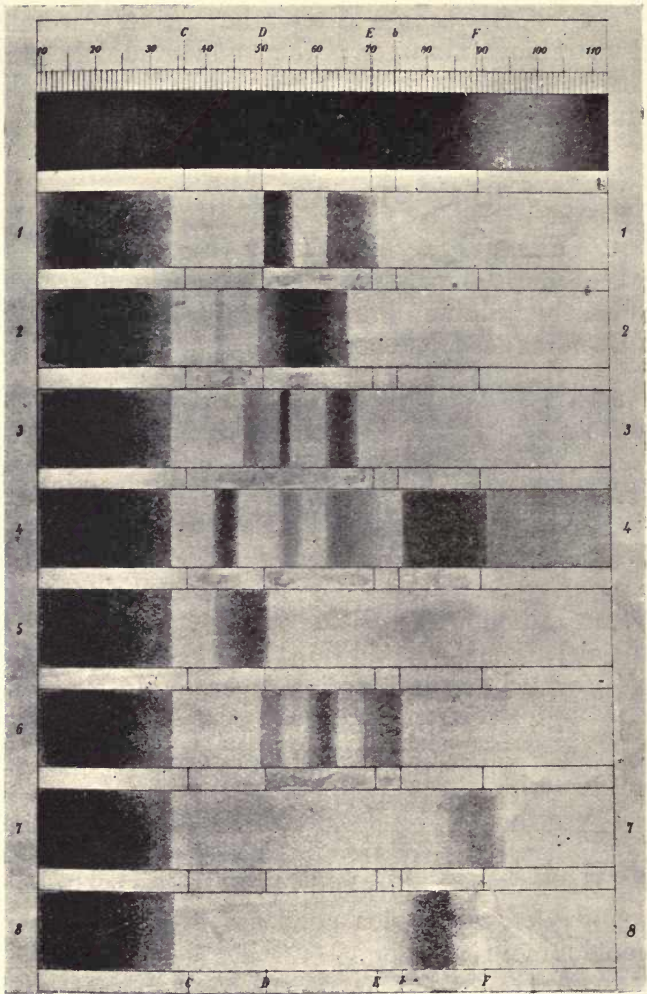


Fig. 72. Spectra of (1) Oxyhaemoglobin ; (2) Haemoglobin ;
 (3) Alkaline Methaemoglobin ; (4) Methaemoglobin in
 Neutral or Acid Solution ; (5) Alkaline Haematin ;
 (6) Reduced Haematin (Haemachromogen) ;
 (7) Urobilin ; (8) Zinc-Urobilin

In *alkaline* solution the acid band disappears, and a faint band on the red side of D takes its place (compare with alkaline haematin).

Reduced by ammonium sulphide, the bands of reduced haemoglobin are got. It differs from oxyhaemoglobin in its chemical reactions by the fact that it is precipitated by basic or neutral lead acetate solution, whereas oxyhaemoglobin is not.

Detection :—

1. In presence of oxyhaemoglobin. Ppt. with basic lead acetate ; filter, decompose the precipitate with carbonate of soda solution ; examine for the bands of alk-methaemoglobin.

2. In presence of urobilin. Proceed in the same way.

3. In presence of bile pigments. Precipitate these by making the solution alkaline with ammonia adding CaCl_2 .

4. In neutral solutions its spectrum is identical with that of haematin in natural solutions (Neubauer and Vogel). Reduced by $(\text{NH}_4)_2\text{S}$, methaemoglobin is changed to reduced haemoglobin and haematin to reduced haematin, the bands of which are easily recognized.

UROBILIN

Frequently occurs in the urine in jaundice instead of bile pigment.

According to Hayem, it is associated with methaemoglobinaemia. Its occurrence in blackwater fever is very common, occasionally before the attacks, but more constantly after the oxyhaemoglobin has disappeared or together with it.

Characteristics :—

1. In *acid* urine a band near F occurs, between 88 and 101.
2. In *alkaline* urine a band between 81 and 95.
3. Make the urine strongly alkaline with ammonia, filter, add ZnCl_2 solution, but not sufficient to form a permanent precipitate.

A green fluorescence occurs, and the much clearer band nearer 'b' than the acid band.

Detection :—

1. If oxyhaemoglobin is present. Precipitate the urobilin with *basic* lead acetate, then acidify the precipitate, when the urobilin goes into solution.
2. If methaemoglobin is present. *Neutralize* the urine with carbonate of soda; precipitate the methaemoglobin with neutral lead acetate. Filter; test the filtrate for urobilin.

BILE PIGMENTS

Where urobilin is present, as in blackwater, the colour of the foam on shaking the urine, the staining of the filter paper, etc., cannot be regarded as satisfactory tests.

Detection :—

1. *Gmelin-Rosenbach Test.*—Filter the urine through filter paper (Swedish). Dry; apply a drop of nitric acid (fuming) to this, a play of colours is got.
2. *Huppert's Test.*—Precipitate the urine with BaCl_2 . Filter; wash the residue off the filter (perforated) with acidulated (H_2SO_4) alcohol. Boil. A bright green colour indicates bilirubin.
3. *Smith's Test.*—To ten c.c. of the urine add two c.c. of dilute tincture of iodine (tincture of iodine 1, alcohol 10). A green ring forms at the junction zone.

BILIRUBIN AND HAEMATOIDIN (IN URINARY SEDIMENT)

1. Bilirubin crystals form yellowish-brown rhomboidal plates or needles.

Easily soluble in CHCl_3 . Gives Gmelin's reaction, green, under the microscope.

2. Haematoidin, dark red in colour or greenish if impure, with nitric acid they give a transient blue.

According to Hoppe-Seyler, however, they are identical.

HAEMATOPORPHYRIN

Occurs in urine as alkaline haematoporphyrin (Fig. 73). In urate sediments a similar form occurs. It is soluble in chloroform, giving bands similar to those of oxyhaemoglobin, but acid converts this into acid haematoporphyrin bands. Solutions have a brilliant red fluorescence. It is found in the urine in toxic conditions, such as chronic sulphonal poisoning. It is precipitated by lead acetate, while oxyhaemoglobin is not.

SUGAR

Before testing for sugar, boil to remove all proteids.

Reduction of copper solution is effected by bile pigments. Reduction occurs also in patients taking salicylic acid, sulphonal, and quinine (Simon), so that it may be necessary to use—

1. *Fermentation Test* or

2. *Phenyl-Hydrazine Test*.—Take a pinch of pure phenyl-hydrazine, ten drops glacial acetic acid, one c.c. of a saturated solution of common salt; add three

c.c. of urine ; boil for three minutes ; cool ; crystals separate out in a few minutes up to one hour. This is an exceedingly delicate test.

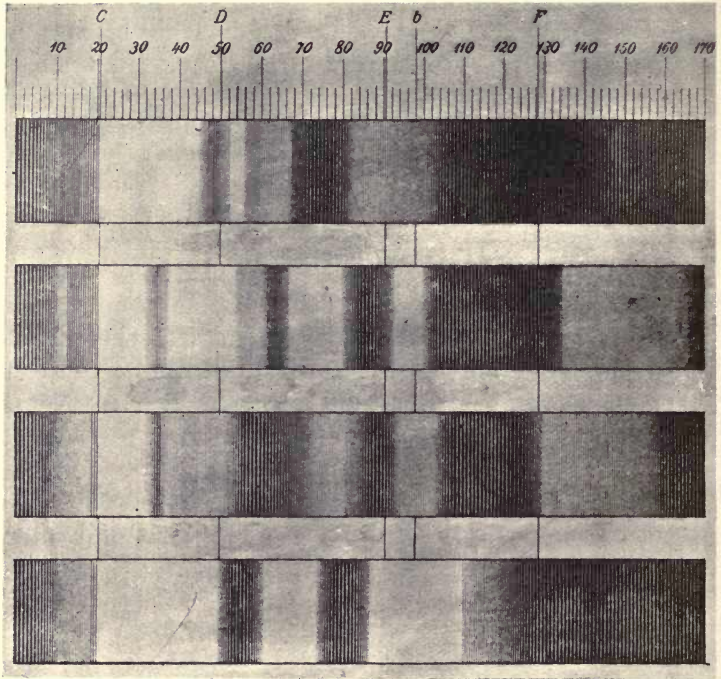


Fig. 73. Spectra of (1) Haematoporphyrin (in acid Solution) ; (2) Haematoporphyrin in Alkaline Solution ; (3) in Neutral Solution ; (4) Haematoporphyrin (urate-sediments)

THE DETECTION OF QUININE IN THE URINE*

The detection of quinine in the urine is of importance in connexion with the property that this

* Kleine. *Zeitschrift für Hygiene*. Bd. xxxviii, H. 3, s. 460.

drug has of inducing attacks of haemoglobinuria (blackwater fever) in patients resident in regions where malaria is especially virulent, and where generally the parasite form is the malignant tertian associated with an extremely high endemic index of native (children) malaria.

Two hundred c.c. of urine are acidified with some drops of sulphuric acid. A spoonful of solid picric acid is then added. The solution is allowed to stand for an hour and then filtered. The solution should be quite clear and should give with a saturated solution of picric acid no turbidity. If there is difficulty in getting a clear filtrate add a trace of egg albumen and filter again. The half-dry residue is then digested in an Erlenmeyer flask with fifty c.c. of 3.0 per cent. soda solution for half an hour on the water bath. Now add sixty c.c. chloroform; shake for two hours in a shaking apparatus. The solution of chloroform is now removed by means of a separating funnel and collected in a weighed flask. The flask should have a long neck to prevent spurting. Evaporate in a water bath and dry at 120°C . The residue is quinine. The experimental error is only one to two per cent.

DETERMINATION OF THE PERIODICITY OF PARASITE DEVELOPMENT

The inspection of a temperature chart is not in itself sufficient to determine the cycle of development of a parasite. Thus, as is well known, a quotidian temperature chart may be produced by a double tertian (simple) or by a triple quartan infection. If then, in the case of the double tertian, we made microscopical examinations at definite intervals for forty-

eight hours, we should find in the blood at any particular time parasites in *two* phases of development corresponding to each cycle. The accompanying chart shews how, in the case of what proved to be the malignant tertian parasite, we were able to establish the cycle of development. We proceeded to make blood examinations at frequent intervals (four hours). We found that at any particular time parasites of various sizes might be found, but by counting several hundred parasites in each film and estimating their size with a micrometer we found that at any particular time there was a preponderance of parasites of one size. Thus, at ten p.m. on the 2nd, there are numerous small forms, *i.e.*, about one-seventh to one-eighth of a red cell in diameter, and it is not till ten p.m. (about) on the 4th that the same condition of blood is found again, accordingly the parasite had a developmental cycle of forty-eight hours (approximately). And, further, we determined the periods taken to develop from small forms to largest forms in the peripheral blood (about eighteen hours) and the disappearance of these and the reappearance of numerous youngest parasites (about thirty hours). So that by determining these three periods we were able to conclude that the parasite was the malignant tertian.

In order then to determine the cycle of a parasite it is necessary :—

1. To estimate the size and percentage of parasites of each size at any particular time, *e.g.*, starting with the onset of the attack.
2. To follow each group to its period of maximum development in the circulation.
3. To estimate the time between this period and the next appearance of young forms.
4. To estimate the time between the appearance

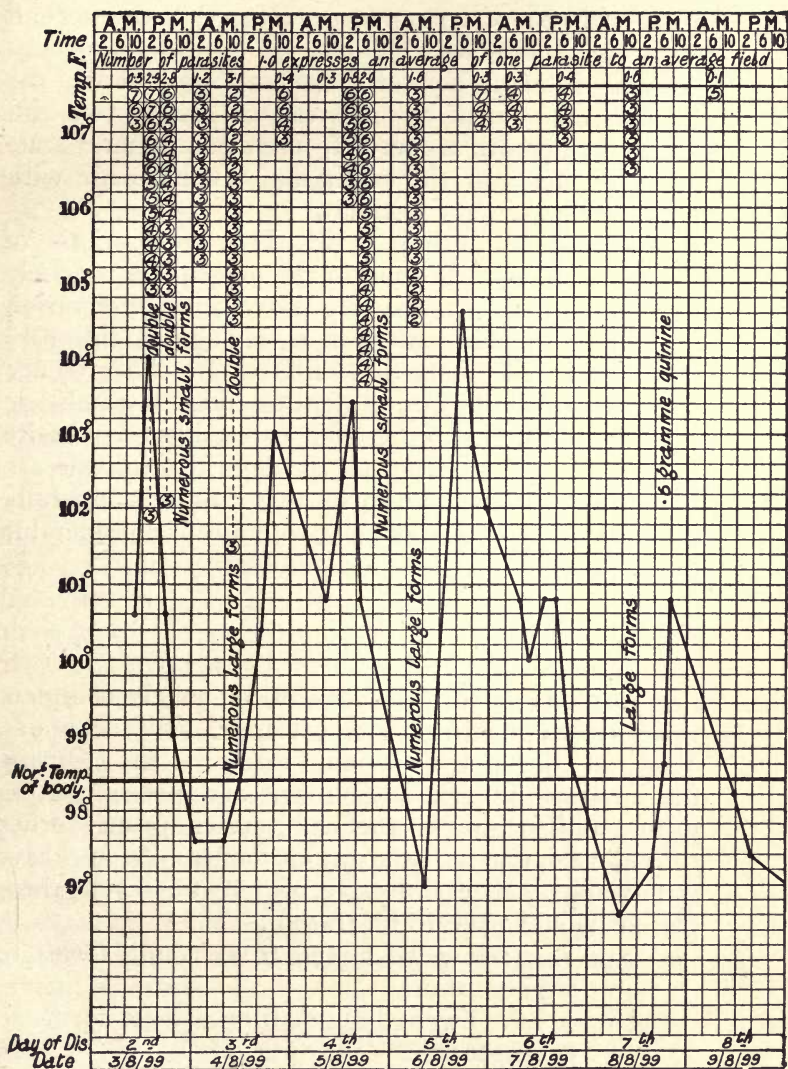


Fig. 74. Illustrating method of determining the developmental Cycle of a Parasite. (The figures within the circles represent the size of the Parasites—thus 7 signifies the Parasite is one-seventh the diameter of a red cell)

of an outburst of young forms (No. 1) and a second similar outburst (No. 4).

The interval between one and four should be equal to the sum of the intervals of periods two and three. It is more accurate to use a micrometer scale for measuring, but the estimation can be made with considerable accuracy without.

If we are dealing with three generations of parasites as in a triple quartan the principle is precisely the same, though it may require careful observation to separate the different groups, though in this particular case the process is facilitated by the presence of segmenting and presegmenting bodies which are easily counted. In order then to establish a parasite cycle, repeated observations at definite intervals are necessary, and also the temperature should be carefully recorded every four hours or two hours as considerable variations may otherwise escape observation.

QUOTIDIAN

Parasites have been described which complete their development in twenty-four hours (about). Thus, at the pyrexia young forms occur. During the apyretic interval large forms and presegmenting forms, and, again, at the next attack young forms, thus developing in twenty-four hours. As we have stated above, to establish accurately this cycle three periods would have to be traced :—

- No. 1. (? Twelve hours) from young forms to largest forms.
- No. 2. (? Twelve hours) from largest forms to young forms.
- No. 3. Twenty-four hours from young forms to young forms.

While some consider that the quotidian temperature is due to the fact that the malignant tertian has a very variable period of development, viz., twenty-four to forty-eight hours, and, in fact, all intermediate times, others consider that with one generation of parasites there is a second accumulation of young forms in sufficient quantity to produce a quotidian attack.

In quotidian fever, due to the malignant tertian parasite, the characteristic febrile attack, with its preliminary pseudo-crisis, is lost. The attack instead of lasting about a day lasts a few hours only, as in the simple tertian, and instead of a pseudo-crisis there is a true crisis, but the young parasites, as in the malignant tertian attack, are still coming into the circulation, and there follows a rise which replaces the apyretic day in the ordinary malignant tertian.

According to Maurer, in the case of the quotidian chart produced *by one generation of malignant tertian parasites* we have the febrile attack produced by the division of the majority of the segmenting forms, and then a fall to normal occurs; when, however, there is a sufficient accumulation of young forms arising from the same generation there is again a rise giving the quotidian chart. As we have seen, the young forms of the malignant tertian parasite persist during the pyrexia. If, however, by any means they are destroyed or cease appearing temporarily during the day of pyrexia, we should get a fall to normal, and then as soon as this inhibiting cause was removed, again a rise, giving a quotidian chart produced by the malignant tertian parasite.

IRREGULAR TEMPERATURES

Besides the typical malignant tertian temperature chart and the quotidian chart, various irregular tem-

peratures may occur, due solely to the malignant tertian parasite. Such charts are not at all uncommon in first attacks in the tropics, and may be followed by charts with regular curves.

The malignant tertian parasite has a developmental cycle of about forty-eight hours, and it seems more likely that these irregular charts are produced by an irregular irruption of young forms into the circulation than that the parasite has a variable time of development. If we suppose that young fission forms exist in the internal organs, which come into the circulation irregularly, then we should have still a constant time of development, but an inconstant time at which the development started. If, however, a quotidian parasite exists, there should be no difficulty, as we have stated above, in determining the fact by a series of measurements at fixed intervals.

ACTION OF QUININE

Action of Quinine on Parasites.—Quinine although it does not prevent fission yet destroys the young ring forms.

As is well pointed out by Marchiafava and Bignami the ensuing attack may still lack nothing in severity, although parasites are exceedingly scanty.

Although this may be considered as the typical action of quinine, yet there are cases, as anybody who has observed really severe cases of tropical fever, *e.g.*, in West Africa, well knows, in which quinine has not always this inhibitory effect.

In such cases the number of parasites may be exceedingly small or even absent, and yet the severity of the symptoms persist. To those cases where with severe symptoms and yet an absence of parasites and to those cases where other factors promote the rapid

disappearance of parasites we shall refer in the succeeding section.

1. *On Young Parasites* (malignant tertian).—When quinine is given at the time of their first appearance in the circulation, the parasites continue in the circulation for a variable period of time depending upon the amount of quinine and probably on other unknown factors. Although parasites are still found yet their growth is arrested, and the outburst is not followed by large forms, presegmenting, and eventually fission forms. It must be noted that quinine may have no such inhibitory effect at all.

2. *On Large Parasites*.—The parasites still go on developing as far as presegmenting and segmenting forms, but generally there is no subsequent production of young forms.

3. *On Presegmenting and Segmenting Forms*.—These are, as we have said, rarely found in the circulation, but if quinine is given at the time that corresponds to this stage, the subsequent effect is that very few young rings appear at the next attack.

QUININE HAEMOGLOBINURIA

Between this phenomenon and blackwater fever there is, in our opinion, practically no difference. It is apparently true that cases of blackwater fever do rarely occur in which no quinine has been previously administered, and in which we have the exciting cause of 'chill,' other drugs, 'exertion,' etc., but it does not effect the position that quinine, *not necessarily* in large doses, is the common cause of this phenomenon.

It occurs only in those who have previously suffered from malaria, and, in fact, there is considerable evidence to shew that it occurs frequently in *direct association* with a malarial infection. It has often been

denied that blackwater fever is malarial at all, on account of the scarcity or frequent absence of parasites, but, as we shall show on page 243, this depends upon when the examination is made. Regarding the haemoglobinuria attack :—

1. The haemoglobinuria follows the administration of quinine after a certain variable interval, two to three frequently, five to six or possibly twenty-four hours.

2. The amount of quinine does not determine whether the haemoglobinuria is slight or severe.

3. After haemoglobinuria has been produced by quinine, a second administration does not necessarily produce a second attack of haemoglobinuria.

These facts clearly shew that it is not the quinine, *per se*, but a condition of blood in the particular malarial patient which is the determining factor whether quinine will produce an attack.

This is further borne out by the well-known fact that the aborigines rarely, if ever, suffer from haemoglobinuria, but it is in Europeans subjected to unnatural climatic conditions and subjected to virulent malaria that the disease is most frequently found.

We would only add, finally, that it is quite illogical to abstain from quinine in malaria, on the contrary, its *adequate* administration would prevent the occurrence of these attacks.

As we have already said, an accurate study of the urine in these cases and in the allied cases of malaria where quinine produces urobilinuria is necessary.

Especially important is the study of the urine and the blood in the prehaemoglobinuric state. It would, of course, involve an accurate study of all possible subjects of the disease, and more especially those who had already had an attack.

Chapter XXIII

BLACKWATER FEVER

Diagnosis.—Attention should be paid to the following points :—

(1) *Haemoglobinuria.*—The colour of the urine may vary from a very light red to a dark porter colour.

(2) *Jaundice.*—Varying from a pale lemon yellow to a deep bronze.

(3) *Constitutional Disturbance.*—Slight, or extremely severe, with a high temperature, vomiting of green bile, sudden anaemia, pain over kidneys and gall bladder, collapse.

EXAMINATION OF THE BLOOD IN BLACKWATER FEVER

1. Note the difficulty in obtaining a full-sized drop of blood.

2. Observe the 'thin' nature of the blood drop, its 'oily' nature, and the difficulty with which it adheres to the slide. These properties are best seen in severe cases.

3. Collect a specimen in a fine pipette and allow the serum to separate. Observe whether the serum is yellow (cholaemia) or reddish (haemoglobin-aemia), using the spectroscope if necessary.

4. To some of patient's serum add 'washed' (p. 225) red cells, (1) of the patient, (2) of a normal person. Observe whether there is any haemolysis, *in vitro*, or microscopically.

5. Determine tonicity of patient's blood (p. 225).

(a) Vincent and Dopter recently determined

the isotonic point of the blood of a patient suffering from chronic malaria, and who had had no blackwater for a month, and found it to be 0·44 per cent. NaCl, *i.e.*, its resistance was less than normal.

(b) One hour after taking quinine, at the beginning of the actual haemoglobinuria, the resistance had fallen, *i.e.*, the isotonic point had gone up to 0·46 per cent.

(c) Four hours after the haemoglobinuria, the resistance had increased up to 0·41 to 0·42 per cent.

(d) Nine days after the haemoglobinuria it had reached the normal.

Haemolytic Action of Quinine.—(1) The amount of quinine hydrochloride (neutral) sufficient to haemolyse 1 c.c. of an emulsion of washed red cells at 37° C., *in vitro*, was 0·001 to 0·00082 gramme in a healthy patient. (2) On the contrary, in the blackwater patient, it was less, *viz.* : 0·0008 to 0·00062, or even during an attack, 0·0005.

Action of Calcium Chloride.—The blood in the above case, with an isotonic point of 0·46, was exposed to the action of calcium chloride (one drop of a ten per cent. solution, to 3 c.c. of the blood cell emulsion). The corpuscles were then washed in 0·075 per cent. salt solution once. The isotonic point was now found to be lower, *viz.* : 0·41, *i.e.*, the resistance to haemolytic agents was raised.

6. Count the red and white cells. The red cells are, as a rule, quite normal in shape.

7. Determine the amount of haemoglobin.

8. Make films every two hours if possible (*as early as possible*), noting accurately the time and temperature at which the films are made.

9. Examine films for parasites; if these are absent, search carefully several large films for pigmented

leucocytes, as these, as also in ordinary malaria, may require long search.

10. Make careful differential counts of the leucocytes, especially when the temperature is falling, as it is then that the mononuclear increase is most marked. When the temperature is raised (*e.g.*, 103° to 105°) the polynuclears may reach ninety per cent.

11. Observe presence of normoblasts, megaloblasts, various abnormal staining reactions, *e.g.*, polychromasia of the red cell, especially during recovery.

12. Make careful blood counts immediately before and after administering quinine when no haemoglobinuria results. According to Panse there may result a blood destruction due to the quinine, which does not shew itself as haemoglobinuria.

Microscopical investigations in this disease are frequently negative as regards malaria parasites, but it is all important when the examination is made, as the following analysis of over one hundred cases microscopically examined shows :—

AUTHOR	Day before Haemoglobinuria		Day of Haemoglobinuria		Day after onset	
	No. of Cases	No. Positive	No. of Cases	No. Positive	No. of Cases	No. Positive
A. Plehn	5	5	5	3	10	2
F. Plehn	0	0	21	18	10	3
Koch	5	5	8	6	6	1
Stephens and Christophers ...	1	1	9	2	16	0
Daniels	3	3	3	1	2	0
Panse	9	8	17	9	20	5
Total	23	22	63	39	64	11
Percentage Positive ...	95·6 per cent.		61·9 per cent.		17·1 per cent.	

In a series of cases examined by ourselves in Nyassaland we found malaria parasites only in 12·5 per cent., but, as we have already shewn, we have two further tests for a malarial infection :—

- (1) The increase in the percentage of large mononuclear leucocytes.
- (2) The presence of pigmented large mononuclear leucocytes.

By using these tests we were able to prove that 93·7 per cent., not 12·5 per cent., of our cases were due to a malarial infection.

Further, in the only case of blackwater fever seen by us *before* the onset of haemoglobinuria, *parasites were present* in abundance; afterwards they rapidly disappeared.

EXAMINATION OF THE URINE IN BLACKWATER FEVER

1. Before the attack (if possible) examine for albumen, urobilin, reducing bodies, etc.
2. Examine so-called 'high-coloured' urines. As a rule these do not shew bile pigment.
3. Examine urine during an attack for methaemoglobin (or haematin), oxyhaemoglobin, urobilin, bile pigment (unusual), bilirubin crystals, haemoglobin casts, granular or hyaline casts, blood cells (rare).
4. Centrifugalize the urine. Examine the clear layer (as in 3), and make films of the sediment.

The sediment may contain hyaline and granular casts stained with haemoglobin. The mass of the sediment, however, consists of masses of haemoglobin of a yellowish-red colour.

UROBILINURIA

As we have indicated elsewhere, the occurrence of urobilin may be an important indication in cases where a susceptibility to quinine haemoglobinuria exists: thus, in Murri's case, a girl had haemoglobinuria eight times between August 3, 1894, and April 6, 1895, following upon the administration eight times of small doses of quinine. From 1895 to 1897, the girl remained well. On March 27, 1897, she was given 0.5 gramme of quinine, to see whether her disposition to quinine poisoning still remained. The result was fever, vomiting of bile, etc., albuminuria, peptonuria, and urobilinuria (not haemoglobinuria).

A. Plehn, in a recent paper, points out a peculiar property of the urine sometimes observed in blackwater cases. On boiling the urine and allowing to stand for some time, a bright purple colour appears.

We have observed that blackwater urines made alkaline with potash, and then boiled produce a purple colour, giving the bands of haemöchromogen (reduced haematin), shewing that the urine itself contained reducing bodies.

Whether Plehn's purple colour is the same we cannot say.

POST-MORTEM EXAMINATION

1. Make smear preparations of spleen, kidney, liver, bone marrow, brain, etc. Examine for parasites and pigmented leucocytes. Parasites are generally absent, but pigmented leucocytes may occur in large numbers in the spleen. Fine pigment is also found in the liver in endothelial capillary cells. (Fig 13).

2. Cut sections, especially of brain tissue, as parasites may be found in the capillaries and nowhere else.

Spleen.—Malarial pigment (melanin) occurs in large mononuclear cells and in giant cells (macrophages). Melanin may also occur in the stroma or even beneath the capsule.

Liver.—Melanin occurs in endothelial cells, and especially in macrophages. Yellow pigment (haemosiderin) occurs in the liver cells, also, to a certain extent, in the same situations as melanin. Apply the iron reaction (*vide* Appendix) to the sections. Haemosiderin gives the blue colour, melanin does not.

Kidney.—Necrosis and desquamation of the epithelium of the convoluted tubes. The straight tubules are blocked with masses of granular matter, staining dark red with eosin. Interstitial nephritis usually not present.

Bone marrow.—Evidence of malarial infection (pigment). Proliferation of normoblasts (cp. p. 49).

LITERATURE

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Chapter XXIV

THE HAEMOCYTOZOA—*Continued*

The haemocytozoa, or endoglobular haematozoa, are divided by Laveran into three genera :*

1. Genus *Haemamoeba*. 2. Genus *Haemogregarina*. 3. Genus *Piroplasma*.

GENUS HAEMAMOEBA

Endoglobular parasites, generally pigmented with two generations, an asexual of multiplication and a sexual of reproduction.

The genus *Haemamoeba* includes the malaria parasites with which we have already fully dealt.

HAEMAMOEBAE IN MAMMALS

1. *H. pitheci*.—In the ourang-utan the young forms resemble malignant tertian. The gametes in form and in their pigment resemble quartan, while schizogony forms resemble those of simple tertian. The red cells are stippled. It is not transmissible to other apes.

2. *H. inui*.—In monkeys, *Macacus cynomolgus* and *M. nemestrinus*; resembles the former parasite but the pigment is very abundant and light yellow in colour. The red cell is not stippled. Segmenting forms with twelve to sixteen merozoites.

* In the imperfect state of our present knowledge this classification is for the present retained.

3. *H. cynomolgi*.—In *Macacus cynomolgi*. The red cell is stippled. Dividing forms have eight to thirteen merozoites.

4. *H. kochi*.—In chimpanzees and monkeys. The forms usually met with are sexual forms. Asexual forms resembling young malignant tertian parasites are rare. Flagellation can be seen in fresh specimens. The parasites in the fresh film are spherical pale bodies containing brownish-yellow pigment. On staining, two types can be distinguished. The male (mikrogametocyte), pale homogeneous blue with much chromatin; the female, deep blue, granular, with little chromatin.

No temperature changes occur in the infected animals. The infection is not transmissible by inoculation (*cp. halteridium*).

Post-mortem.—The spleen is pigmented, the capsule thickened. Pigment also occurs in the marrow.

5. [*H. bovis*].—Parasites in the blood of cattle, described by Kolle in South Africa. They have a general resemblance to malaria parasites, but are quite distinct from *Piroplasma bovis*. They produce remittent fever and severe anaemia, but not haemoglobinuria. Kolle also describes pigment in red cells (independently of parasites), but what this means is not clear.*

6. *H. vassali*.—In the squirrel (*Sciurus griseimanus*); the larger forms are pigmented. The red cells are unchanged. Male and female gametes also occur.

7. *H. (?) talpae*.—These parasites in the mole more or less completely fill the red cell, a mere shell of

* Kolle. *Zeitschrift für Hygiene*. 1398.

which is left. The parasite is oval, the protoplasm stains a light blue, and the nucleus shews a loose mesh-work of chromatin. We have often observed these bodies in the blood of moles, but have considerable doubts as to their parasitic nature and as to their inhabiting red cells.

HAEMAMOEBAE IN BATS

1. *H. murinus*.—In *Vespertilio murinus* shews ring forms, medium forms, male and female gametes and sporulating forms. The spores are twenty to twenty-two in number and of an irregular angular shape. The sporulating forms are found almost entirely in macrophages in the liver. This parasite is pigmented and causes enlargement but no stippling of the red cell.

2. *H. melanipherus*.—In *Miniopterus schreibersii* has a general resemblance to the quartan parasite. Gametes are the forms most commonly found, though young and medium forms also exist. Sporulating forms are unknown. The red cell is unchanged. During hibernation, only gametes are found in the blood.

3. *H. vesperuginis*.—Occurs in the blood of various species of bats. It is unpigmented. Minute spindles, rings, large forms and Piroplasma-like forms occur. It produces considerable anaemia and degenerative changes in the red cell. It possibly is a Piroplasma. It is possible that these parasites are conveyed by wingless flies, of the family *Nycteribiidae* which occur on bats. In *V. capensis*, the South African Serotine bat, haemamoebae also occur.

4. *H. monosoma*.—In *Vesperugo*, *sp.*, in Annam : sexual forms only are known.

HAEMAMOEBAE IN BIRDS

I. *H. relictæ* (*Proteosoma grassii*).—Discovered by Grassi in the blood of birds in Italy. It causes a fatal disease in the Hungarian partridge (*Perdix cinerea*). In certain regions sparrows and goldfinches are commonly infected. Sparrows are frequently infected in India. In Africa numerous small birds were examined by us, but *Proteosoma* was never found (only *Halteridium*). Transmission from one bird to another by inoculation is readily effected. Canaries are extremely susceptible. Pigeons, among other birds, are immune. Birds that have recovered from an infection have acquired a well-marked immunity against a subsequent inoculation. The parasite is closely allied to the malaria parasite, and is especially suitable for the study of the exogenous mosquito cycle.

Endogenous Cycle (Fig. 75).—The parasite in its earliest stage is unpigmented. Coincident with growth a grain or two of pigment appears, and the characteristic property of the parasite shows itself, viz., the displacement of the nucleus of the red cell, so that the nucleus may take up a position at right angles and away from the normal one. All stages of development up to segmenting forms are found in the blood at the same time, so that no cycle of development can here be followed; nor is there any intermission in the clinical symptoms (temperature, etc.) of infected birds.

Exogenous Cycle.—Besides the asexual, sexual forms occur in the blood. They are spherical hyaline bodies of two varieties, characterised in stained specimens by the same general differences which distinguish the male and female gametes of the malaria parasite.

(i) The male cell possesses a mass of compact chromatin and faintly staining protoplasm.

(ii) The female cell possesses but little chromatin, but stains deep blue (Romanowsky).

Flagellation.—(i) This can be observed in a simple wet film preparation. Make stained specimens according to the method given on p. 41, or

(ii) Use artificial serum (bird's serum, one part; salt solution, 0.6 per cent., nine parts), and to this add a trace of bird's blood. Make a series of hanging drops in moist chambers. Dry, fix, and stain, from time to time, according to stage of development, observed microscopically.

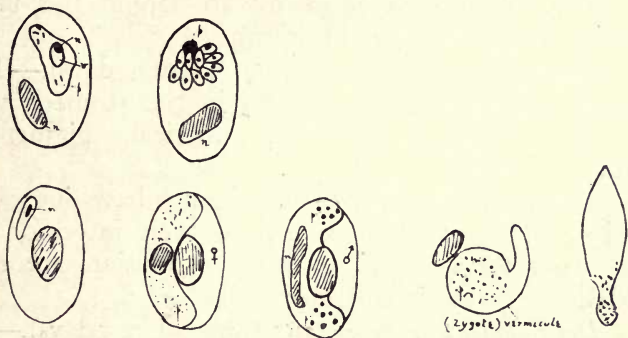


Fig. 75. (Upper line) *Proteosoma* shewing medium-size Parasite and Segmenting Form. (Lower line) *Halteridium* young form Female and Male Gametes, and Vermicule

Further stages of development (vermiculi) have not been observed on the slide.

Development of Vermiculi.—(i) Determine what species of *Culex* is the suitable one for the process of development. *C. nemorosus* was used by Koch, in Italy. *C. fatigans* is also a carrier.

(ii) Collect the *Culex* that have fed on sparrows, etc., roosting at night in trees. The *Culex* can be

caught in large numbers in shaded drains, under bridges, in outhouses, etc., and excellent material is in this way easily got. Identify the species of *Culex* that is infected.

(iii) For the method of feeding mosquitoes on birds' blood, *vide* p. 95.

Twelve to fifteen hours.—Vermiculi in all stages of development are found in the stomach; a conical projection arises from the fertilized gamete. This gradually elongates, forming a long, curved, oval body, the complete vermiculus. The protoplasm is vacuolated, and a nucleus (chromatin) is readily shown by staining (Romanowsky).

The proteosoma vermiculi are larger and more slender than those of halteridium.

Development of Zygotes (one or two days).—The vermiculi have disappeared, but in the stomach wall are now found transparent, spherical, pigmented bodies.

Three to four days.—The zygotes have increased in size, and sporoblasts appear in their interior. In the larger forms, signs of further division are seen (striation), formation of sporozoites.

Development of Sporozoites (nine to ten days).—By this time the sporozoites have reached the salivary glands. Somewhat earlier they can still be found amidst the thoracic muscle. Earlier still, they can be pressed out of the ripe oocysts in the stomach wall. The sporozoites occupy chiefly the middle lobe of the gland (Koch).

Black Spores are found in the larger zygotes. They also occur free in the thoracic region (or, possibly, in the gland substance). They are brownish-black, curved, sausage-shaped bodies, suggesting a mycelial nature. It is believed by Grassi that they

are degenerated sporozoits, as they are found within the large sporoblast cysts. We have, however, found them in or about the salivary glands in *Myzomyia rossi*.

According to Ed. and Et. Sargent, a mosquito can infect two consecutive birds but not a third. All attempts to obtain infection by the progeny of infected mosquitoes failed.

The following two parasites resemble *Proteosoma* :

2. *H. majoris*.—In the great tit (*Parus major*), the number of merozoits is sixteen.

3. *H. vaughani*.—In an American blackbird (*Merula migratoria*) with only four merozoits.

4. *H. danilewskyi* (Halteridium). — Occurs almost exclusively in the blood of 'passerine' birds. Pigeons are very commonly infected, also sparrows, finches, parrots, Java sparrows, and many other birds. It remains to be seen whether there exists one or very many species.

The parasite is characterised by its peculiar curved halter shape, embracing the oval nucleus of the red cell without any displacement of the latter (Fig. 75). Young forms are occasionally seen, but whether these are young sexual or asexual forms is not determined. Segmenting forms and those corresponding to an asexual cycle, as in *proteosoma*, are unknown.*

Two varieties of parasites, the male and female gametes, are easily distinguished.

(i) Note that the male gamete has a clear hyaline appearance. On staining (use *undiluted* Romanowsky) a central large mass of chromatin is distinguished, while the protoplasm is a *faint* blue. Five or more oval pigment grains are placed generally at either extremity.

* They have recently been described in the lung blood.

(ii) In fresh specimens the female gamete is finely granular, and the pigment is frequently scattered throughout. On staining, a *small* amount of chromatin is shewn, while the protoplasm takes on a *deep* blue colour.

Flagellation.—Select an infected bird that shews numerous gametes in each field. Proceed in the same way as in *Proteosoma*. The gametes first become spherical and then escape from the red cell. The pigment of the male gamete displays violent movement, and in a few minutes four to eight flagella are extruded. The motion of these is at first so rapid that they cannot be distinguished, but the corpuscles in the neighbourhood are seen moving. In a few minutes one or more breaks off, and if, fortunately, a female gamete is in the same field, the loose flagellum (mikro-gamete) can be seen entering the female. The pigment of the latter shews active movements at this stage.

Vermiculi.—The formation can readily be observed on the slide. A conical projection forms at one point of the fertilized gamete (copula). This elongates slowly and gets curved, forming an egg-shaped or spindle-shaped mass. The conical portion eventually separates, leaving behind the remains of the cell with the pigment. The vermiculus is thus at first unpigmented, but later again it is pigmented (Koch). In the fresh specimen the protoplasm appears vacuolated, and has a nucleus which is readily stained by Romanowsky stain.

Note that the vermiculus (or ookinet) shews forward, rotatory, and peristaltic motions.

Development in the Fly.—The brothers Sargent have shewn that infection in the case of *H. columbae* of the pigeon is transmitted by a Hippoboscid, *Lynchia*

(= *Olfersia*) *maura*. These flies infest pigeon houses. The incubation period in the pigeon may be as long as thirty-eight days. The cycle in the fly has not yet been traced. It remains to be seen also whether Hippoboscids transmit halteridia of other birds.

Post-mortem.—Pigment is found in the kidney, intestine, bone marrow, liver, and especially the spleen. The brain, on the contrary, is almost entirely free from it.

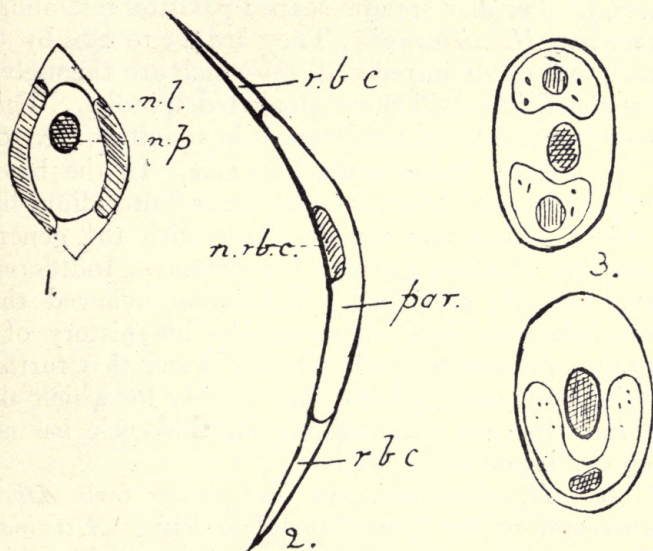


Fig. 76. (1) *H. smithi*; (2) *H. neavei*; (3) *H. testudinis*

It is probable that the halteridia of all birds are not of the same species. Inoculation from one bird to another is extremely difficult, if not impossible. This may be due to the fact that the parasites in the blood are all sexual forms.

7. *H. smithi*.—In smears from the liver of the turkey (*Meleagris gallopavo domestica*). The parasite

is oval 14 by 8μ , or elongated 25 by 5μ . It is pigmented. The cells in which these parasites lie are oval or spindle-shaped, with the nucleus divided into two parts one on each side of the parasite. They are probably leucocytes, and the parasites leucocytozoa.

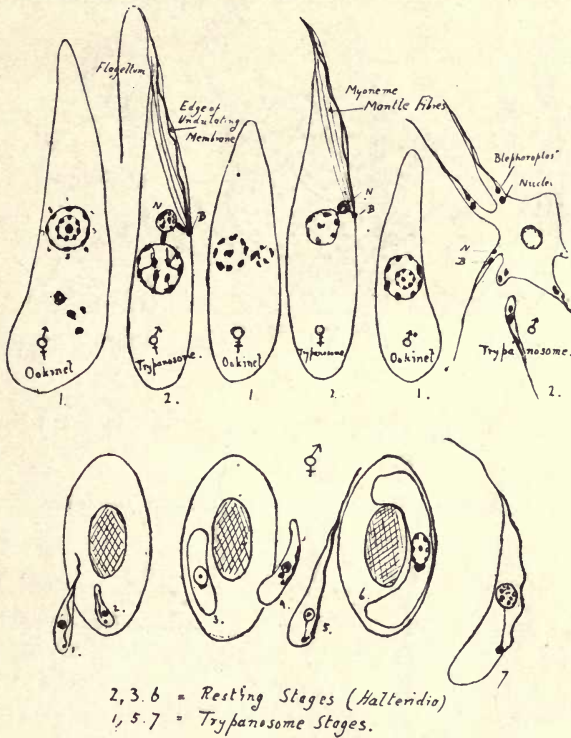
It is somewhat doubtful if these parasites are the cause of the disease of turkeys (Fig. 76).

8. *H. neavei* (Balfour, 1896).—In the blood of the Abyssinian helmeted guinea-fowl (*Numida pitlorhyncha*). Peculiar spindle-shaped parasites resembling somewhat *H. ziemanni*. They are 15 to 20μ by 5μ broad, and occur in red cells (?) which are themselves compressed into still more elongated spindles. They shew male and female differences in staining (Fig. 76).

9. *H.* (= *Haemoproteus*) *noctuae*. In the blood of the little owl (*Athene noctua*) occur halteridium-like parasites. Male and female forms with the general characters of other gametes are present. Indifferent forms are also described. Schaudinn believed that these halteridia were stages in the life history of a trypanosome also found in the blood and that further development occurred in *Culex pipiens* by which also the infection was transmitted, but this cycle has not been confirmed (Fig. 76A).

10. *H. ziemanni*.—In the little owl *Athene noctua* and in the grey Congolese hawk (*Asturinala monogrammica*). The nature of the cell in which these parasites occur is, according to some, a red cell, according to others a leucocyte. Full grown forms are long spindle-shaped parasites which have displaced to one side the nucleus of the host cell. They consist of periplast or sheath shewing striations, ectoplasm and endoplasm and nuclear structures. The female type is characterised by deeply staining endoplasm with many chromatin granules and vacuoles, and by

a small amount of nuclear matter. They measure 55 by 10μ about. The male stains much less deeply and has more abundant nuclear matter. Quite young



2, 3, 6 = Resting Stages (*Halteridia*)
1, 5, 7 = Trypanosome Stages.

Fig. 76A. Upper figure: shewing the three kinds of ookinet and the three trypanosomes developed from them in the mosquito's stomach. Lower figure: shewing the development of the indifferent trypanosomes into *H. noctuae* in the blood (after Schaudinn)

forms consisting of a nuclear dot surrounded by a little protoplasm also occur, and also a variety of stages between these and full-grown adults.

Schaudinn has described the development of these parasites in the stomach of *Culex pipiens*, where they eventually become trypanosomes and finally minute spirochaetes. Infection then takes place during biting

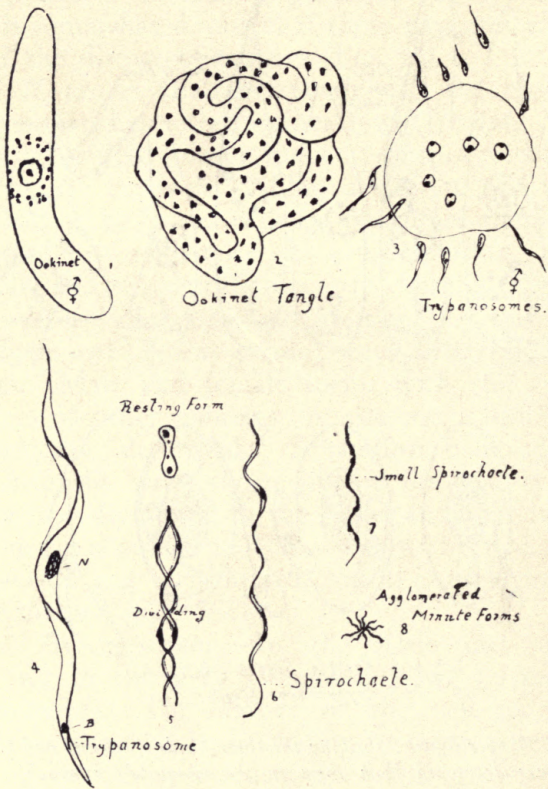


Fig. 76B. Showing development of the ookinet of *H. zimmermanni* into trypanosomes and then spirochaetes, in the mosquito (after Schaudinn)

and after again passing through a trypanosome stage they infect the blood cells again. This has not been confirmed, and it must be borne in mind that owls are

not uncommonly infected with trypanosomes and also spirochaetes.

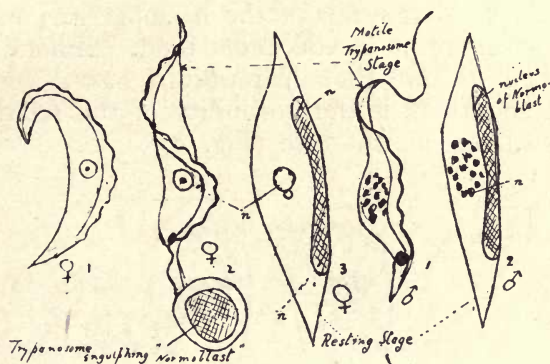


Fig. 76c. Development in the blood of *H. ziemannii* and change to resting forms (after Schaudinn)

II. *H. lovati* in the red grouse (*Lagopus scoticus*).

HAEMAMOEBAE IN TORTOISES

1. *H. metchnikowi*.—Found in the blood of *Trionyx indica*, or *Chitra indica*, a large fresh-water tortoise, in many Indian rivers. All adult specimens of this tortoise from the Jumna were infected.

The parasite resembles *H. danilewskyi* (halteridium), in that two forms are easily distinguished in the blood—(1) a hyaline form with large pigment grains, staining very slightly with methylene blue; (2) a granular form, with fine pigment, staining deeply with methylene blue. These forms correspond to the male and female gametes respectively. In one of Simond's figures it is interesting to observe a male and female gamete in the *same* red cell, which, so far as we know, has never been observed in the case of *H. danilewskyi*. But besides these pigmented forms there are

also found unpigmented forms, which have the typical gregarine look, that is to say, curved, worm-like bodies. The exact relationship of the haemogregarine to the haemaebae forms is not understood. Simond, however, points out that halteridium has a vermicule stage, and there is the possibility of the relationship being similar in this case (Fig. 77).

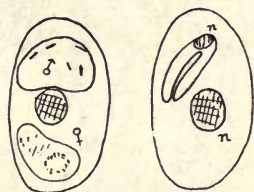


Fig. 77. *H. metchnikowi*, Gametes and Vermicule

2. *H. testudinis*.—In the blood of *Testudo pardalis*. Three forms occur (1) Unpigmented oval forms 3μ in diameter; (2) Pigmented reniform parasites 10 to 12μ in diameter. Two may occur in the same cell; (3) Pigmented horseshoe-shaped forms 20 by 8μ . Male and female forms occur (Fig. 76). In this respect and in the possession of pigment it resembles *H. metchnikowi* and it probably should be classed as a haemamoeba.

HAEMAMOEBAE IN LIZARDS

1. *H. ('Haemocystidium') simondi*.—In the blood of a tree-living gecko (*Hemidactylus leschenaultii*) in Ceylon. Has a general resemblance to *H. metchnikowi* but is larger, *displaces* the nucleus, and has more pigment. Male and female forms occur shewing characteristic differences in staining. There is no vermicule form.

HAEMAMOEBAE IN BATRACHIANS

1. *H. ranarum*.—In *Leptodactylus ocellatus*, Saô Paulo. Apparently a leucocytozoon. It is 30 to 40 μ by 10 to 15 μ . The nucleus 7 to 10 μ , and another stained body 2.5 to 4 μ . Sexual differences have not been observed.

ADDENDUM

Ectoglobular Parasite in Man.—Found by the brothers Sergent in Algeria. They are vermiform in appearance about 40 μ by 1 to 1.5 μ in size, pointed at the ends. Apparently a nucleus occupies the middle third of the body (Fig. 78). The parasites disappeared regularly from the blood about 6 p.m. The patient in whose blood they were found on two different occasions, suffered from night sweats and nausea.

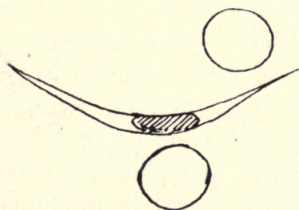


Fig. 78. *Ectoglobular parasite in Man*

Chapter XXV

GENUS HAEMOGREGARINA

The haemogregarines are *unpigmented* parasites mostly found in the blood of cold-blooded vertebrates, but also in that of a few mammals. They are endoglobular parasites; but with a free vermicule stage. The great majority attack the red blood corpuscles; but certain of the mammalian forms attack the leucocytes. They are, so far as is known, non-pathogenic, and they cannot be transmitted by inoculation from one animal to another. Schizogony not infrequently takes place in the cells of some viscus, liver, kidney, bone marrow, etc. The sexual cycle of development has only been described in a few cases and cannot yet be said to be definitely known.

According to some authors three kinds of haemogregarines exist in the blood, thus Prowazek, in *H. platydactyli*, describes:—

Indifferent Forms.—The nucleus is rich in chromatin and stains very deeply. The protoplasm is dense and filled with red staining matter (Romanowsky). These forms frequently leave one red cell and enter another.

Female Forms.—Broader than the former, and frequently found expressed from the cell.

Male Forms.—Slender, with a chromatin rich nucleus, the protoplasm staining a light blue. They often contain a blepharoplast. The males are often vacuolated. The red cells are often bent by the movements of the parasite, and in stained films are boomerang or banana shaped.

Technique.—Examine the blood of warm and cold-blooded animals. For schizogony make sections

or thick films of various viscera. Examine for cystic stages, with a low power lens, especially the liver, or in the case of snakes, the lungs.

HAEMOGREGARINES OF MAMMALS

1. *Hg. balfouri*.—In the jerboa (*Faculus* spp.) four forms occur. (1) Endoglobular, sausage-shaped parasites, about 6 by 2μ in size, with a voluminous nucleus. The red cells are deformed and may be reduced to a mere trace. (2) Free forms, of the same appearance as the former. They are most readily found in liver smears. (3) A

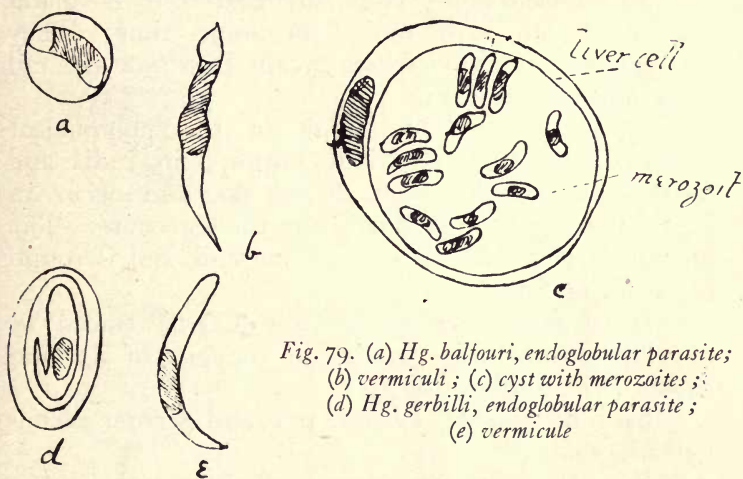


Fig. 79. (a) *Hg. balfouri*, endoglobular parasite; (b) vermiculi; (c) cyst with merozoites; (d) *Hg. gerbilli*, endoglobular parasite; (e) vermicule

typical motile vermicule, 15μ long (rarely found). (4) Schizogony occurs in the liver—the final stage is that of a cyst, formed by the remains of the liver cell, containing merozoites. The cysts measure 23 by 17μ (but some are much longer and others smaller), containing as many as thirty merozoites (Fig. 79).

The mode of development is unknown.

2. *Hg. gerbilli*.—In the red cells of *Gerbillus indicus*, an Indian field rat. Parasites are generally numerous. It is non-pathogenic. Two forms occur.

(i) *Endoglobular*.—They lie in sharply defined oval cavities limited by a 'cytocyte,' probably derived from the red cell. The parasite possesses a short tail sharply flexed upon the body. In stained specimens a nucleus is visible just before the bend of the tail. The tail portion stains deeper than the rest of the parasite and contains a number of chromatin granules. The red cells are anaemic and enlarged, and become oval, corresponding to the shape of the parasite.

(ii) *Vermicules*.—These are best seen in blood that has been kept moist for some time. They resemble the encysted forms except that now the tail is straightened out (Fig. 79).

3. *Hg. canis*.—Occurring in the polynuclear leucocytes of dogs, especially puppies, in India and Assam. They are about 12 by 6μ , and occur in a capsule or cytocyte derived from the leucocyte. The nucleus is generally situated at one end, and is round or oval (Fig. 80).

Development.—One of us (S. R. C.) has traced the development of this parasite in the gut of *Eu. sanguineus*, the Indian dog tick.

(i) The parasites become free, and become vermicular in shape.

(ii) The vermicule enters a cell of the gut and proceeds to divide, giving rise to a slender and stout form.

(iii) Conjugation of these takes place, resulting in a zygote.

(iv) These grow in size and eventually in them develop sausage-shaped sporozoites.

(v) How these get out of the tick and infect dogs is unknown.

4. *Hg. gerrardi*.—A leucocytozoon in the polynuclear leucocytes of dogs in the Federated Malay States. It closely resembles, but is probably different from the former as in this case the nucleus has generally an U-shape and is not oval or spherical.

5. *Hg. felis-domestici*.—In bazaar cats in India. The infection is scanty. It occurs in polynuclear leucocytes and differs from *Hg. canis* in not lying in a cytocyst.

6. *Hg. funambuli*.—A leucocytozoon in the mononuclear leucocytes of the palm squirrel *Funambulus pennantii* (with five pale dorsal stripes) of Kathiawar, India. It does not occur in the Madras palm squirrel (*F. palmarum*).

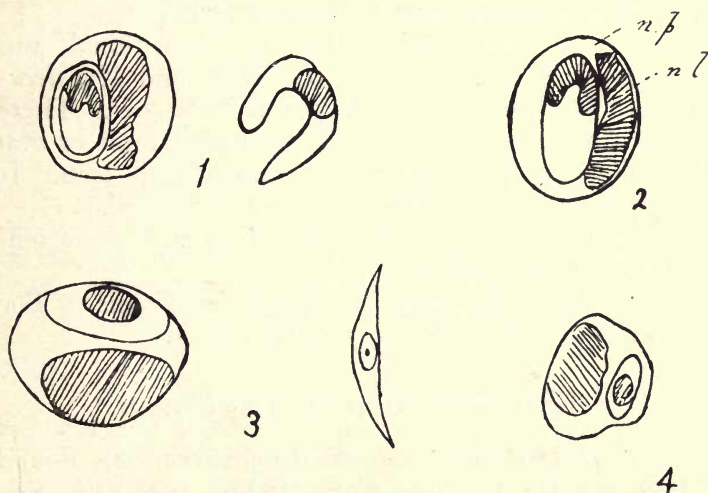


Fig. 80. (1) *Hg. canis*. (2) *Hg. gerrardi*. (3) *Hg. funambuli* endoglobular and free forms. (4) *Hg. ratti*

Endoglobular Form.—10 by 5 μ . It is not contained in a cytocyst. It is broad at one end and narrow at the other, this being bent so as to form 'a tail.' The nucleus, centrally placed, is large, occupying about a third of the volume of the parasite (Fig. 80).

Vermicules.—In the blood of heavily-infected squirrels. They are spindle-shaped and measure 14 by 3.5μ . They exhibit active serpentine motion, and attach themselves to red cells and revolve with remarkable velocity.

Pathology.—As far as is known, the squirrels do not suffer from the infection, but there is a remarkable change in the leucocyte values. There is a distinct mononuclear leucocytosis varying with the degree of infection, e.g., 10,000 per mm.³, instead of 4,000 normally; the relative value is correspondingly increased, viz., 80 per cent. of mononuclears in severe infections instead of 25 to 30 per cent. normally.

Mode of Development.—Is unknown.

7. *Hg. ratti* (Adie).—In the transitional and mononuclear leucocytes of *Mus rattus* and *Mus decumanus*. Its dimensions are about 11.7 by 5.6μ . There is no cytocyst. The nucleus is quadrilateral or oval according to the aspect from which it is seen. It occupies about a third of the length of the cell.

In all cases examined so far, *T. lewisi* has accompanied it in the blood.

8. *Hg. bovis*.—In the ox in Abyssinia. The parasites are club-shaped, 7-10 by $1.6-2\mu$.

HAEMOGREGARINES IN BATRACHIANS

1. *H. ranarum* (= *Lankesterella ranarum*). Found in the blood of *Rana esculenta* (edible frog) (Fig. 81). This species includes, according to Laveran, two species *H. princeps* and *H. monilis*, described by Labbé. The cycle of development is, according to Hintze, as follows :—

(i) *Asexual Forms or Schizonts*.—These are endoglobular, four to eight μ in length. Increase

in size takes place, and eventually they become spherical and divide into a number of segments (schizonts). According to some observers segmenting forms are only found in the spleen.

(ii) *Sexual Forms*.—Free in the plasma, twelve to fifteen μ long. These are male and female, and are characterized by the same general differences as other gametes; the male mikrogametocyte is slender and finely granular; the female makrogametocyte is fat and coarsely granular.

(iii) A mikrogamete in the form of a small mass of chromatin separates off and fertilizes the (now) makrogamete.

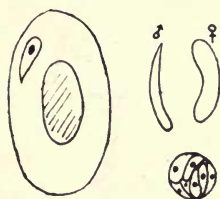


Fig. 81. *H. ranarum* (or *Drepanidium ranarum*) young form, Gametes free in the Plasma, and Fission forms in Spleen. (Partly after Minchin)

(iv) A zygote results, which is at first motile. This becomes encysted as the

(v) Oocyst, which is found in the *epithelial cells* of the intestine. This passes out eventually in the faeces of the frog. Sporoblasts are formed as in the malarial cycle, and from these result

(vi) *Sporozoits*.—These would gain access to a fresh frog which had swallowed an oocyst.

According to Billet development takes place in leeches of the genus *Helobdella*.

2. *H. splendens* (= *Dactylosoma splendens*).—
Found in the blood of *R. esculenta*.

The following forms are figured by Labbé (Fig. 82) :—

- (i) Amoeboid forms.
- (ii) Forms resembling in shape a finger-glove.
- (iii) Segmenting forms as in *Haemamoeba relicta* (Proteosoma).

The protoplasm contains no pigment but refractile granules.

This differs from the typical development of haemogregarines, and it is probable that its position requires revision. According to Hintze, it is a variety of *H. ranarum*.



Fig. 82. *H. splendens*.—Adult form with
Refractile Granules

3. *H. magna*.—Described by Grassi and Feletti in *R. esculenta*. Minchin thinks it may be the makro-gamete of *H. ranarum* or *H. monilis*.

Occurs most commonly in an U form. The liver is especially rich in parasites. The free vermicules measure 24 to 30 μ . The parasites cause extreme hypertrophy of the nucleus and red cell (Fig. 83). Peculiar forms of degeneration or resistance also occur in which there exists only a faintly stained bluish mass, 11 by 6 μ , containing a mass of chromatin granules.

'*Cytamoeba bacterifera*.'—In frogs not uncommonly curious rod-shaped bodies are found lying in a vacuole in the red cell. When these occur further search will show cysts filled with these rod-like bodies. Originally described as protozoan parasites, they are considered by Laveran to be bacterial in nature.

4. *H. berestneffi*.—In frogs in Bombay. Resembles *H. magna*. Encapsulated and free vermicules occur. The latter have the nucleus near the blunt anterior end.

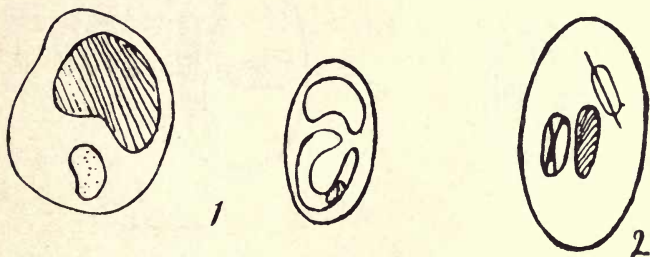


Fig. 83. (1) *Hg. magna*; (2) '*Cytamoeba bacterifera*'

5. *H. theileri*.—In *R. angolensis* in the Transvaal. Large haemogregarines, 15 to 17 by 5 to 6 μ (Fig. 84). (1) They are oval, spindle-shaped, or with one end slightly recurved. Some lie in a cavity which may either be a cyst or only a cavity in the red cell. (2) Vermicule free forms, 24 by 4 μ , also occur. The red cells are much altered and their nucleus may be divided into two parts.

6. *H. neireti*.—In *R. mascariensis*. They are oval in shape. 16 to 21 by 11 to 14 μ . The nucleus is elongated and set at right angles to the axis of the haemogregarine. The largest forms have one end slightly recurved (Fig. 84).

7. *H. tunisiensis*.—In *Bufo mauritanicus*. (i) Vermicule form. The two limbs of the vermicule are equal; the vermicule is encysted; at one end of the cyst is a deep staining mass. According to Billet there is a second cyst included in the first. This second cyst shows stippling with Romanowsky, but the red cell is not hypertrophied. (ii) Elongated halter-form, 18μ by $4-5\mu$. Parasites are especially abundant in the liver.

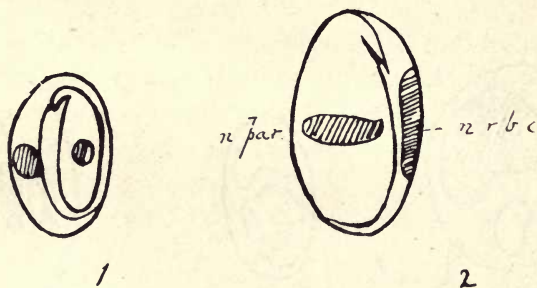


Fig. 84. (1) *Hg. theileri*; (2) *Hg. neireti*

Other species are *H. encapsulata* in *R. tigrina*, and *R. limnocharis* in India. *H. tritonis* in *Triton cristatus*; *H. clamatae* and *H. castebianae* in frogs in U.S.A., and *H. riedyi* in a Californian salamander (*Batrachoseps attenuatus*).

HAEMOGREGARINES IN SAURIANS

1. *H. lacertarum* (= *Karyolysus lacertarum*) (Fig. 88).—Found in the blood of *Lacerta agilis*, *L. muralis*, and *L. ocellata*.

The parasite has a more compact form than some of the other haemogregarines. They exert a marked action on the red cells, which become much enlarged

and anaemic, and, as the name of the species implies, a disintegrating action on the nucleus is one of its effects. The nucleus is either pressed to the side or broken up into fragments.

The parasite in its endoglobular stage becomes surrounded with a *cytocyct*. Some of these cysts divide up into about a dozen *macromerozoites* (Fig. 88), while others divide up into twice as many or more *micromerozoites*. Corresponding to these we have free forms in the liver, twelve by three μ and eight by two μ respectively. It is said to be transmitted by ticks in the larval and nymphal stage.

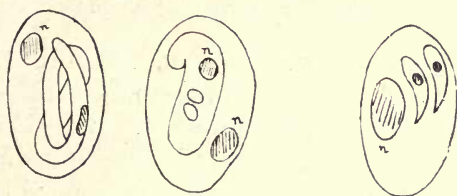


Fig. 85. (1) *H. mesnili*, shewing characteristic looped *Vermicule* ; (2) *H. laverani*, shewing characteristic hooked *Vermicule* and two bright *Granules* (after Simond) ; (3) *H. bigemina* in Blood of Blennies (after Minchin)

2. *H. lacazei* (= *Haemocytozoon claratum*).—In the blood of lizards. The vermicules have a peculiar shape (Fig. 88). Here also cyst formation has been described in the spleen by Labbé.

3. *H. sergentium*.—In a lizard, *Gongylus ocellatus*. Has a destructive action on the nuclei of the red cells. They flatten and elongate, and also fragment. Parasite is reniform, 15-18 μ by 5-6 μ . The protoplasm has numerous granules.

4. *H. curvirostris*.—In *Lacerta ocellata* var. *pater*. Differs from the previous one. (1) The vermicule form

destroys the nucleus. (2) The encysted form becomes embedded in the nucleus.

5. *H. mabuiae*.—In a lizard *Mabuia vittata* from Berberi, 15 by 5.5μ , has a destructive action on nucleus. Resembles closely *H. sergentium*.



H. curvirostris

Fig. 86. Shewing disintegrating action on nucleus

6. *H. varani*.—In *Varanus*, spp., in India and Africa. The parasite is endoglobular and occurs in three forms. (1) Small round or oval forms, $3-4\mu$, with a relatively large nucleus. (2) Elongated forms, 7-10 by $3-4\mu$, pointed at one end, the nucleus also being elongated. (3) Vermicules (endoglobular), 14 by

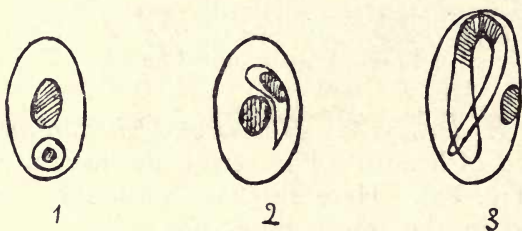


Fig. 87. *Hg. varani*

3μ . One end is recurved. The length of this end may be quite short, or as long as the other limb (Fig. 87).

7. *H. borreli*.—In *Varanus griseus*, resembles the former.

Other species are *H. biretorta* in *L. ocellata*, *H. platydactyli* (Billet) in *Platydactylus mauritanicus*, *H. psammodromi* in *Psammodromus algirus*, *H. mabuiae* in *Mabuia vittata*, *H. erlichi* in *Varanus exanthematicus*, and *H. macroscinci* in *Macroscincus coctaei* (Cape Verd Islands).

HAEMOGREGARINES IN CHELONIANS

1. *H. stepanowi*.—It is found in the tortoise, *Cistudo europaea*. This may be taken as the type haemogregarine. It presents the following forms (Fig. 88):—

(i) Reniform parasites, ten to fourteen μ long. Curved and thickened at each end, granular, non-pigmented. Intermediate forms occur between this and the next developmental stage.

(ii) Vermiculate forms, also endoglobular, but after examining a fresh specimen of blood for some time, free forms are seen thirty to forty μ long and three to four μ broad. These are actively motile, and constrictions can be seen travelling down their length during the motion.

(iii) Young forms and segmenting forms are not seen in the circulation. These are found in the liver. The segmenting forms are at first endoglobular, but later free. They occur as ovoid forms, ten to sixteen μ long by four to six μ broad, shewing as many as six nuclei (chromatin masses). The protoplasm finally segments and there is formed

(iv) An actively amoeboid young form (merozoite).

The spores that are found in the kidneys of tortoises belong, according to Laveran, not to the haemogregarine at all, but are those of a *Myxosporidium* (*M. danilewskyi*).

According to Siegel infection is transmitted by a leech (*Placobdella catenigera*). The ookinets reach the oesophageal glands and give rise to numerous sporozoites, which pass out during the process of biting.

2. *H. mesnili*.—In the blood of a tortoise, *Emys tectum* (Fig. 85).

Amoeboid forms, reniform, and vermicule forms occur. Besides these, free merozoites, but their origin is obscure. The form of the vermicule is characteristic at one stage of its development.

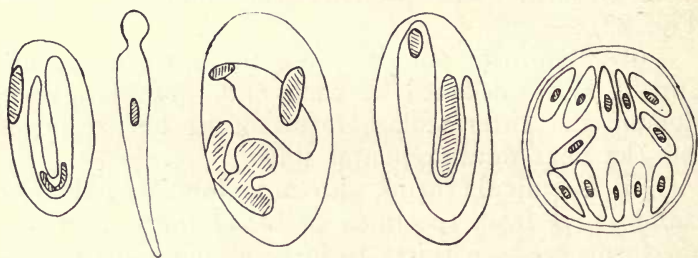


Fig. 88. *H. stepanowi*, Endoglobular and free Vermicules ;
H. lacertarum, shewing disintegration of Nucleus of
 Red Cell ; *H. lacazei*, *H. lacertarum*, Cyst
 with Makromerozoites

3. *H. laverani*.—In the blood of Indian tortoises, *Cryptopus granosus*. Similar forms occur to those of the last species. The vermicle is characterized by a blunt hook-like appendage, and the presence of two bright granules. The parasite is endoglobular in all its stages (Fig. 85).

4. *H. mauritanica*. — In *Testudo mauritanica*. Resembles *H. stepanowi*. Two forms occur : (i) very granular, smaller forms, with two large refractile granules at each end ; (ii) larger, uniformly pale forms. In stained specimens the smaller forms appear oval or reniform, with a nucleus transversely placed. The

nucleus of the red cell is displaced. The larger forms have at one of the poles a pale mass, staining with difficulty. Division forms are found in the liver.

5. *H. bagensis*.—In a tortoise (*Emys leprosa*) in Tunis. (i) Young stage does not exceed threequarters the length of red cell. (ii) Vermicule stage encysted. The cyst does not stain. Nucleus variable in position, always central according to Billet. (iii) Large oval forms. (iv) Division forms in liver (Fig. 89).

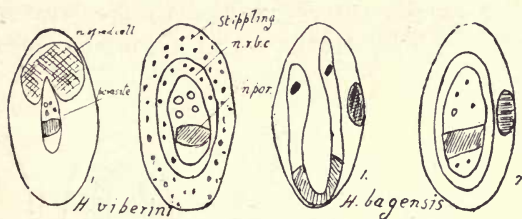


Fig. 89. *H. viberini*. (1) young parasite ; (2) the parasite is encysted in the nucleus, the cyst and the red cell show stippling (Romanowsky). *H. bagensis*. (1) vermicle ; (2) encysted stage

6. *Hg. stepanowia*.—In a fresh water tortoise (*Damonia reevesii*). Closely resembles *H. stepanowi* but differs in the following points :—(i) One limb of the vermicle is quite short. (ii) The nucleus of the vermicle is in the longer limb, not at the bend. (iii) The free vermicle is shorter and thicker (18-20 by 5μ) than in *H. stepanowi*. (iv) The nucleus of the red cell is displaced but not hypertrophied as is the rule in the case of *H. stepanowi*, whereas in *H. stepanowia* it is displaced and hypertrophied. Multiplication forms occur in the liver. (Fig. 90)

7. *Hg. rara*.—In a freshwater tortoise (*Damonia reevesii*). It is crescentic or banana shaped, 15 by 2-3 μ .

It either occupies a lateral position in the red cell or a polar one with much displacement of the nucleus (Fig. 90). The nucleus occupies about two-thirds of the cell. Free forms also occur.

8. *Hg. nicoriae*.—In an amphibian tortoise (*Nicoria trijuga*) in Ceylon. Young parasites are reniform. These eventually become bent on themselves in the typical vermicule way. Their length is then 10μ . They displace the nucleus of the red cell. Specific differences are hard to define.

Other species are *Hg. billeti* in *Trionyx stellatus*. *Hg. labbei* in *Platemys* sp. and *Clemmys elegans*, N. America.

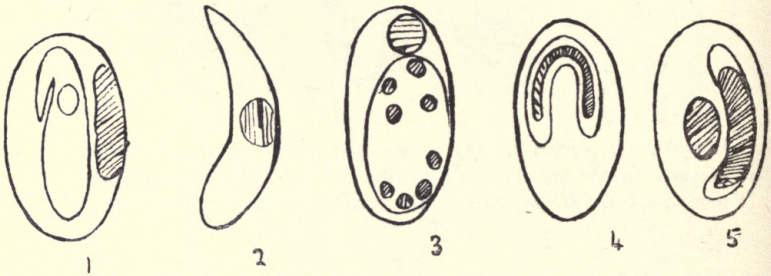


Fig. 90. (1-3) *Hg. stepanowia*; (4-5) *Hg. rara*

HAEMOGREGARINES IN FISH

Haemogregarines and trypanosomes are often found together in saltwater fish, but although trypanosomes are common in freshwater fish, haemogregarines are not.

1. *H. bigemina*.—Discovered by Laveran in the blood of blennies. A vermicule form occurs free in the plasma.

The endoglobular parasite divides by simple binary fission (Fig. 85).

2. *H. bothi*.—In the brill, *Bothus rhombi*, 10 by 2μ . Nucleus $3-4\mu$ in length. *T. bothi* occurs in the same fish.

Other species are :—

H. blanchardi in *Gobius niger*, Brittany; *H. callionymi* in *Callionymus dracunculus*; *H. cotti* in *Cottus bubalis*; *H. delagei* in *Raja* sp.; *H. flesi* in *Flesus vulgaris*; *H. gobii* in *Gobius niger*; *H. laternae* in *Platophrys laterna*; *H. lignieresi* in an eel, Buenos Ayres; *H. platessae* in *Platessa vulgaris*, Brittany; *H. simondi* in *Solea vulgaris*; *H. quadrigemina* in *Callionymus dracunculus*.

HAEMOGREGARINES IN OPHIDIANS

1. *H. viperini*.—Or *Karyolysus viperini*. The parasite bores into the nucleus, and, eventually, encysts there (Fig. 89).

Other species are :—

Hg. bungari in *Bungarus fasciatus*; *Hg. colubri* in *Coluber aesculapii*; *Hg. crotali* in *Crotalus confluentus*; *Hg. joannoni* in *Macroprotodon cucullatus*; *Hg. mirabilis* in *Tropidonotus piscator*; *Hg. mocassini* in *Ankistrodon piscivorus*; *Hg. najae* in *Naja tripudians*; *Hg. pythonis* in *Python reticulatus*; *Hg. tropidonoti* in *Tropidonotus stolatus*; *Hg. viperini* in *Tropidonotus viperinus*; *Hg. zamenis* in *Zamenis hippocrepis*; *H. brendae* in *Psammophis sibilans*; *H. cantlei* in *Eryx conicus*; *H. lübei* in *Corellas cooki*; *H. mansonii* in *Zamenis flagelliformis*; *H. pococki* in *Python molurus*; *H. refringens* in *Pseudaspis cana*; *H. rarefaciens* in *Coluber corais*, var. *couperi*; *H. samboni* in *Vipera aspis*; *H. selig-*

manni in *Lachesis mutus*; *H. serpentium* in *Eunectes murinus*; *H. shattocki* in *Python spilotes*; *H. terzii* in *Boa constrictor*; *H. wardi* in *Coronella getula*.

HAEMOGREGARINES IN CROCODILES

H. cataphracti in *Crocodilus cataphractus*, W. Africa; *H. crocodilinarum* in *Crocodilus frontatus* and *Alligator mississippiensis*; *H. hankini* in *Gavialis gangeticus*.

Chapter XXVI

GENUS PIROPLASMA

Piroplasma. *—(= *Babesia*) are intra-corporal parasites, frequently pear-shaped as the generic name implies. They do not produce pigment, and multiply in the blood by dividing into two, and are transmitted by ticks, the transmission being sometimes hereditary; *i.e.*, the mother tick feeding on a sick animal transmits the disease to some stage of her progeny, or the transmission is only from stage to stage, *e.g.*, from larva to nymph or nymph to adult.

1. *P. bigeminum*.—This disease of cattle, perhaps best known as Texas fever, is world-wide in its distribution. Infection may be latent with few or no symptoms. The acute form is characterised by: (1) High temperature; (2) Haemoglobinuria in about eighty per cent. of cases, which, as in other forms of *Piroplasma*, may cease after some days; (3) Icterus often absent; (4) Anaemia, often extreme; (5) Muscular palsies, staggering gait and other nervous symptoms; (6) Constipation followed by bloody diarrhoea is not uncommon; (7) Death in a week or less; (8) The mortality is from sixty to eighty per cent. In the benign form of the disease there is anaemia, generally an absence of haemoglobinuria, and the duration is about a fortnight.

Blood Examination.—Obtain blood by pricking the snout or a small vein in the ear. Generally one per

* Those *Piroplasmata* shewing bacillary forms are placed by some authors in a new genus *Theileria*; so that we should then have *T. mutans*, *T. annulata*, *T. equi*, *T. parva*, *T. cervi*.

cent. of the corpuscles are infected, or in severe cases as many as ten per cent. Besides typical pear-shaped parasites, round and amoeboid forms occur. Free parasites also are not uncommon in severe cases. Cattle shewing emaciation, staring coat, etc., often shew parasites scantily.

Post-mortem.—The tissues are oedematous and icteric. The spleen and liver are much enlarged. The kidneys are oedematous and haemorrhagic. The lymphatic glands are oedematous and haemorrhagic as in other forms of piroplasma affection. The serous membranes of various organs shew petechiae. The kidneys may have fifty per cent. upwards of red cells infected. Parasites are also numerous in the capillaries of the heart, choroid plexuses, pia mater and brain.

Culture.—Miyajima states that by adding a little defibrinated blood to ordinary bouillon kept at 20° to 30° C., trypanosomes develop, and further, that these give rise to piroplasmosis on injection.

Transmission.—(1) Is easily effected by intravenous or subcutaneous inoculation; the blood of recovered animals is also infective.

(2) The following ticks are known to be carriers. The adult takes infection, the larva gives infection :—

M. annulatus (*vide* p. 324), in America, Africa, etc.

M. australis, in Australia.

I. ricinus (= *reduvius*), in Europe.

P. bovis.—According to some observers this European form differs from *P. bigeminum*: (1) The parasites are plumper; (2) The pears are smaller; (3) The disease is more benign.

Development in the Tick (Koch).—The parasites leave the red cell and become rather long and club-shaped (Fig. 91). At the clubbed end there is a large

round chromatin mass, and the protoplasm projects as a circlet of rays (1). Forms occur in which two club-shaped parasites have stuck together in their long axis or 'copulated' (2). All these rays are eventually withdrawn, and from the second to third day spherical forms of increased volume occur (3).

On the third day structures appear whose relationship to the previous forms is at present unknown. They

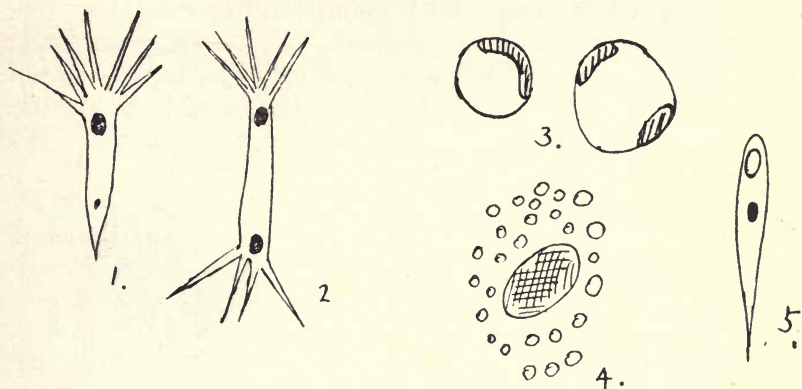


Fig. 91 Development of *P. bigeminum* according to Koch

consist of *immense* numbers of amoeba-like piroplasma forms, surrounding a large nucleus possibly that of a body cell (4). The chromatin in these parasites consists of scattered granules. The parasites separate from the heaps and grow, and the chromatin condenses. They now have a characteristic club shape (5). These forms are seen also in the eggs of ticks. The developmental cycle has not been further traced by Koch, but cp. *P. canis*.

2. *P. parvum*.—Is found in cattle on the East coast of Africa, etc., and a similar, if not identical,

parasite in India and Japan. The symptoms differ in the following respects from those caused by *P. bigeminum* :—

(1) Anaemia is only slight ; (2) Haemoglobinuria is wanting ; (3) It is *not* transmissible by inoculation ; (4) Cattle immunized against *P. bigeminum* are susceptible to *P. parvum* ; (5) The mortality is very high ninety per cent. upwards. The incubation period is about a fortnight. The duration after the appearance of the first symptoms is about ten days.

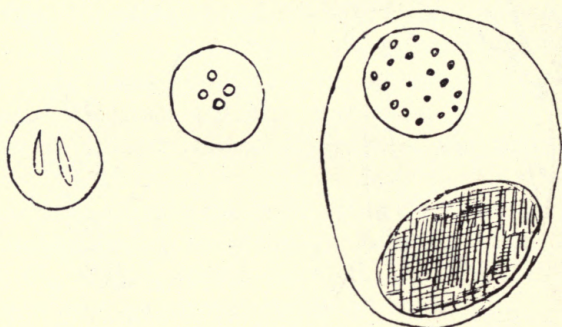


Fig. 92. *P. parvum*. Bacilliform, cross forms, and intracellular protoplasmic masses

Blood Examination.—(1) In the early stages of the disease bacilliform parasites and *minute* rings are found in great abundance ; (2) Later large forms and pears occur scantily ; (3) The arrangement of parasites in the form of a cross is characteristic ; (4) The occurrence of protoplasmic masses (in size from one to three red cells) containing numerous small chromatin particles, in the endothelial cells of the spleen and lymphatic glands (and occasionally in the blood) is also characteristic (Fig. 92).

Post-mortem.—(1) Great oedema of the lung occurs in about a third of the cases ; (2) The lymphatic

glands are enlarged and haemorrhagic; (3) Infarcts occur in the liver, lungs and kidneys. These changes are mainly due to injury caused by the collection of large masses of parasites.

Transmission.—(1) The disease is not transmissible by inoculation. *Eu. appendiculatus*, the brown tick; and *Eu. simus* are known carriers. Larvae which have fed on infected animals transmit the disease in the nymphal stage only. Nymphs which have fed on infected animals transmit in the adult stage only. The transmission is accordingly not hereditary. The blood of recovered animals is not infective as in the case of *P. bigeminum*.

Life history of *Eu. appendiculatus* with three hosts, *i.e.*, changes after the larval and nymphal stage.

Eggs on grass,	According to tempera-	
hatch in	ture and moisture	... 28 days.
Larvae remain on		
grass till they		
find a host.		
On cattle.	Host No. I. Take in-	
	fection	3-4 days.
On grass.	Dormant, first moult	
	takes place	21 days.
Nymphs on cattle.	Host No. II. Give in-	
	fection and take infec-	
	tion	3-4 days.
On grass.	Dormant, second moult	
	takes place	18 days.
Adults on grass.		
On cattle.	Host No. III. Give in-	
	fection. Copulation.	
	♀ drops off	4 days.
♀ on grass.	Egg-laying begins in ...	6 days.
	(Some thousands laid.)	

Development in the Tick.—*P. parvum* passes through similar changes to *P. bigeminum* as far as the forms depicted in Fig. 91, but the amoeba-like forms (Fig. 91 (4)) have not been seen.

3. *P. annulatum* (Dschunkowsky and Luhs).—In cattle in Transkaukasias and Egypt.

Symptoms.—(1) Acute form: daily rises of temperature to 41° for one to two weeks. Temperature then remains high. Pulse one hundred and twenty, thread-like. Respirations thirty-six. Haemoglobinuria seldom. Convulsions. Death. (2) Chronic form: characterised by icterus and progressive weakness and great anaemia.

Blood Examination.—Acute form: over ninety per cent. of the red cells are infected. Bacillary and ring forms occur. Chronic form: ten to forty per cent. of cells are infected, and only cocciform or punctiform parasites are found.

Pathology.—Extensive haemorrhages in most organs. In the abomasum (fourth stomach) occur characteristic haemorrhagic ulcers. Cp. *P. parvum*.

Transmission.—(1) Susceptible cattle can be infected by inoculation. (2) The carrier is *M. calcaratus*, a variety, according to Dönitz, of *M. annulatus*.

4. *P. mutans* (Theiler).—In cattle in the Transvaal, in the blood together with *P. bigeminum*. Differs from this by the fact that it produces *minute* bacillary and 'cross' forms as is the case with *P. parvum*, but is not the same as this though morphologically very similar; as it is inoculable. *P. annulatum* is separated by its morphology and pathological effects. In order to distinguish from *P. parvum*, several blood examinations must be made; this form increasing rapidly in numbers, while *P. mutans* is always scanty.

Transmission.—By inoculation of blood.

Similar associations of *P. bigeminum* and bacillary

forms (? *P. mutans*) have been found by one of us in cattle in Madras (Plate III), by others in Japan, in Dutch East Indies, and in Indo-China (Annam).

5. *P. equi*.—Causes biliary fever in the horse. It occurs in Europe, Africa, India and no doubt elsewhere. The chief signs are (1) Haemoglobinuria; (2) Intense icterus; (3) Fever; (4) Paresis of hind quarters. The disease takes an acute or chronic course.

Blood Examination.—Rings, amoeboid, flagellate, pear and bacillary forms occur. Free forms are rare. Laveran considered the frequent occurrence of four pear-shaped parasites in a group as characteristic of this *Piroplasma*. Further, in horses that have a second attack, peculiar willow-leaf forms are found.

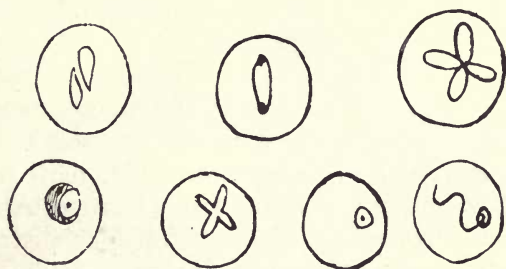


Fig. 93. *P. equi*. Various forms

Post-mortem.—There is great enlargement of the spleen and lymphatic glands. The gut is in a state of catarrh. The kidneys anaemic and soft. Parasites are very numerous in the spleen.

Transmission.—(1) By inoculation; (2) *Eu. evertsi* (the red-leg tick) is a known carrier; larvae and nymphs that have fed on infected animals transmit in the adult stage. The blood of immune animals is infective.

Life history of *Eu. evertsi* with two hosts :—

Eggs.	Hatch on the ground (a variable period)	31 days.
Larvae.	Attack a horse (first host), <i>take</i> infection, moult, while still on the horse, and then become	3-4 days.
Nymphs.	(a) Remain on horse and also <i>take</i> infection in this stage...	16 days.
	(b) Drop on to grass, remain dormant, and then moult and become in	24 days.
Adults.	(a) On grass	
	(b) On horse (second host), <i>give</i> infection, copulate, females suck blood and fall to the ground.	

Piroplasmata occur also in the male ass and zebra, but whether identical with *P. equi* remains to be seen.

6. *P. canis*.—The cause of ‘malignant jaundice’ of dogs. Has been recorded from Europe, Africa and India, but probably has a much wider distribution. The disease takes an acute or chronic course, and may occur in a latent form with few symptoms.

Acute Form.—Is characterised by (1) a high temperature except in young dogs where the temperature may be sub-normal; (2) Marked constitutional disturbance, prostration, anorexia; (3) As a rule haemoglobinuria; (4) Less frequently icterus; (5) After a day or two intense anaemia, with a pipe-clay coloured tongue, pallid gums, and white sclerotics; (6) In very acute cases paresis or paralysis of the hind legs. Death in a few days or in the case of very young dogs in twenty-four hours.

Chronic Form.—Follows the acute attack, when the dog does not die or quickly recover. It is characterised by (1) Extreme anaemia ; (2) Emaciation ; (3) Weakness and anorexia. Haemoglobinuria and icterus are not seen unless, as sometimes happens, an acute attack supervenes after partial recovery. The temperature is variable. All grades of severity of attack are seen, and the types acute, chronic and latent cannot be very clearly defined.

Recovered animals remain infective for a long time, and shew scanty parasites.

Blood Examination.—Snip the extreme point of the ear with a sharp scissors, or in large dogs shave the posterior border and prick with a needle.

Parasites appear first about the fifth or sixth day after subcutaneous inoculation, and earlier in the case of intravenous inoculation. After the bite of infected ticks they may be seen as early as the fourth day or as late as ten, fifteen or twenty days after. They are abundant in the acute disease, except after a crisis, but scanty in the chronic. They may be abundant at the outset though there are few symptoms.

Intracorpuseular forms are (1) Pyriform or oval ; (2) Round ; (3) Irregular (amoeboid), and many shew pseudopodia which may be short and thick or very fine resembling flagella (Plate III).

During growth the parasite is amoeboid, and commonly they stretch themselves nearly across the corpuscle. Division occurs either by the parasite, when in an extended position, becoming constricted in the middle ; or by an amoeboid form dividing into two pyriform halves, each half carrying with it a portion of the chromatin. In this way result two, four, eight, sixteen and even thirty-two parasites in a corpuscle. Single and double forms are always the

most common, but four, eight, and sixteen-forms are frequently seen, especially at the crisis.

Free forms are derived from the intracorpuscular forms, and are usually arranged in groups of four, eight, or sixteen forms. After leaving the cell parasites stain more deeply and sometimes appear slightly larger. In fresh films they exhibit more or less rapid movements and a vibratile action of the fine end. A flagellum-like process is sometimes present in this position, but can rarely be made out in stained films. The chromatin of a parasite consists of (1) A dense mass, the nucleus proper, which is always present; (2) A ragged lighter staining extension of this, which can generally be made out in all well-stained specimens; and (3) A minute punctiform mass, not always present, the so-called blepharoplast.

Changes in the blood are profound, and consist of (1) Reduction in red cells to 2,000,000 or less; (2) Reduction in haemoglobin to a less extent; (3) Leucocytosis, the number of cells reaching 50,000 or more as against the normal number of 7,000 to 8,000; (4) Appearances of nucleated red cells and rapid formation of new corpuscles.

Post-mortem.—Parasites may be more numerous in the organs than in the peripheral blood, but are not always so. Thus while the number of corpuscles in the peripheral blood may be one to three per cent., in the capillaries of the heart there may be seventy per cent.; in the lung, fifty per cent.; kidneys, forty per cent.; brain, eighty per cent.; spleen (trabecular veins), fifty per cent. The parasites are mostly small and spherical. The changes in the organs are most marked in the chronic cases. Ecchymoses occur in various organs, and according to Nocard all changes are due to great dilation of the capillaries by blood cells full of parasites.

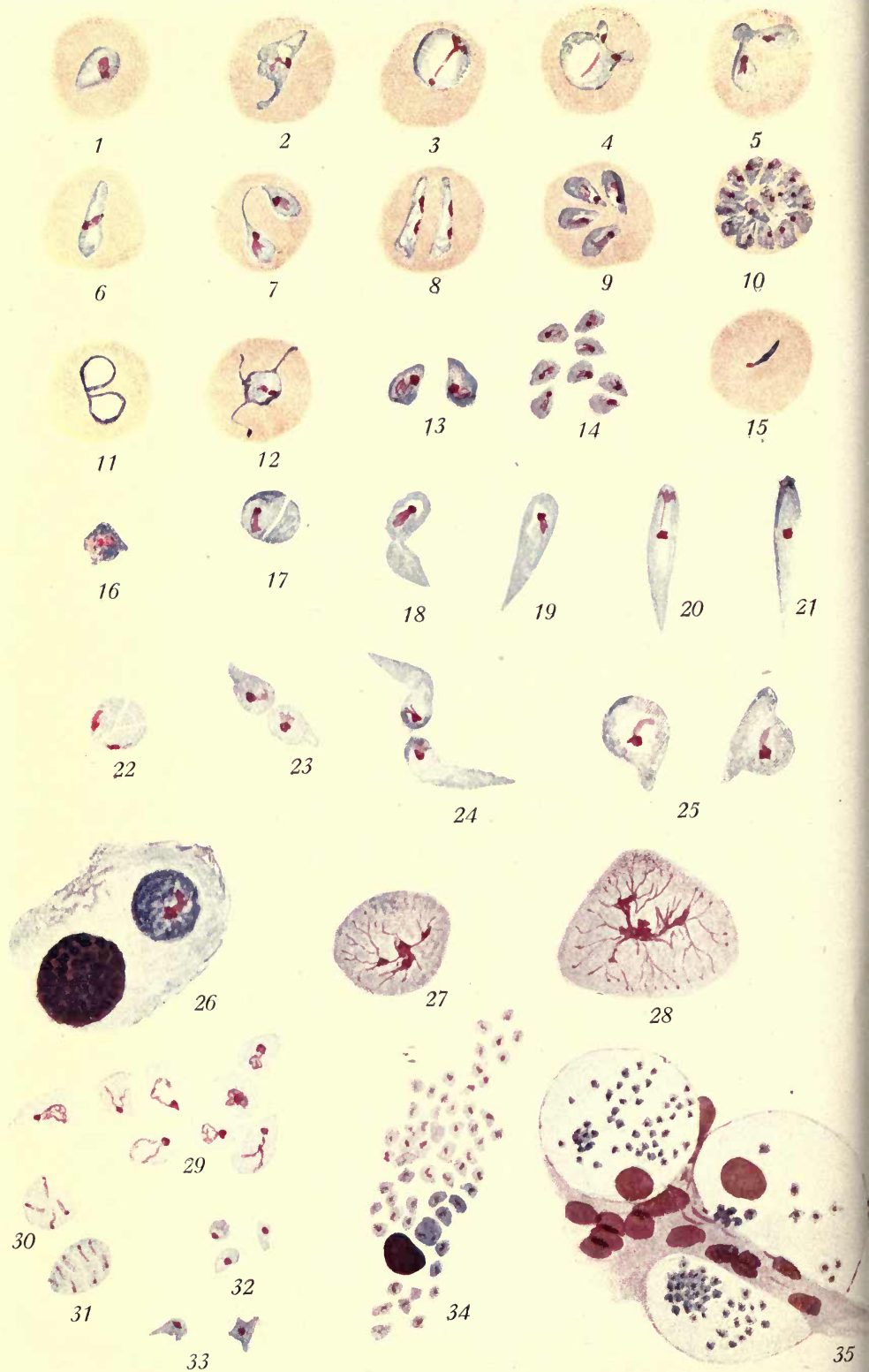


PLATE III

Piroplasma canis

- Fig. 1-5.—Stages in the growth and fission of *P. canis*.
Fig. 6-7.—An alternative method of division after formation of elongate form of parasite.
Fig. 8.—Two elongate parasites about to undergo fission.
Fig. 9.—Group of four parasites in a cell.
Fig. 10.—Group of sixteen parasites in a cell.
Fig. 11.—Clear refractile type of parasite with peripheral chromatin.
Fig. 12.—Form with flagellum-like amoeboid processes.
Fig. 13.—Free forms resulting from dissolution of a cell containing two large forms.
Fig. 14.—Free forms resulting from the dissolution of a cell containing eight forms.
Fig. 15.—Bacillary form of *Piroplasma* (in blood of cattle). *P. parvum*.
Fig. 16.—Early stage in development of *P. canis* in the gut of the tick.
Fig. 17.—Later stage—large bodies with achromatic line.
Fig. 18.—Early stage in formation of club-shaped body resulting from last.
Fig. 19.—Immature club-shaped body.
Fig. 20.—Immature club-shaped body shewing portion of chromatin passing forward to form disk.
Fig. 21.—Fully-developed club-shaped body.
Fig. 22.—Similar stage to 17, but about to form double bodies.
Fig. 23-24.—Later stages of 22.
Fig. 25.—Club-shaped bodies in the tissues undergoing change into 'zygote.'
Fig. 26.—'Zygote' embedded in tissue cell of nymph fed on infective blood.
Fig. 27-28.—Developing zygotes.
Fig. 29.—Bodies formed by fission of a zygote (sporoblasts?).
Fig. 30.—Stage in division of a 'sporoblast.'
Fig. 31.—Division of a 'sporoblast' into sporozoites.
Fig. 32.—Sporozoites.
Fig. 33.—Sporozoites shewing amoeboid processes.
Fig. 34.—Cell of embryonic tissue packed with masses of sporozoites.
Fig. 35.—Two large cells in process of becoming two acini of the salivary gland, containing sporozoites.

The urine is acid, contains haemoglobin (? methaemoglobin) red cells and, not uncommonly, casts and bile pigment. The tissues are more or less pallid, and there may be icterus more or less pronounced.

The spleen is enlarged. The liver is enlarged and often fatty. The kidneys may be normal, or may in haemoglobinuric cases be greatly congested. In old standing cases they may shew few or no changes, or may be large and pale. The bone marrow is foetal in character. The urine may contain haemoglobin or be dark yellow in colour, but even in acute cases it is often clear and free from blood pigment. In chronic cases it is normal in appearance.

Transmission.—*H. leachi* is a known carrier in Africa. Takes infection in adult stage, gives infection in subsequent adult stage. *Eu. sanguineus*, the most widely spread dog tick of the world, is a carrier in India. Takes infection in adult stage, gives infection in following nymphal and adult stage.

Corresponding to this we have the following cycle of development :—

Development in the Tick (Christophers).—(1) The parasite enlarges in the gut of the tick and becomes a motile club-shaped body, which then leaves the gut and penetrates an ovum becoming in the substance of this a 'zygote'; (2) This zygote increases in size and breaks up into 'sporoblasts,' which are found disseminated in the tissues of the *larva*; (3) These sporoblasts further divide up and accumulate as 'sporozoites' in the salivary glands of the *nymph*; (4) Sporozoites accumulate also in the salivary glands of the *adult* tick. They resemble the parasite in the blood except that they are much smaller. In infected ticks they are present in immense numbers (Plate III).

Cultivation.—Prepare a number of test tubes

containing each $\frac{1}{2}$ c.c. of normal salt solution. When parasites are abundant in the blood, chloroform a dog and take blood from the heart. Defibrinate by shaking up in a sterile bottle with pieces of glass, wire, etc. To each tube of salt solution add $\frac{1}{2}$ c.c. of defibrinated blood. Keep at about 27° . Examine next day. Stellate forms are described by Kleine similar to those seen by Koch in ticks (p. 281). These subsequently become spherical. Fülleborn, on the contrary, describes flagellates in culture.

7. *P. ovis*.—Occurs in Europe, Africa and West Indies. The disease is characterised by (1) Intense anaemia, the number of blood cells falling from eight to one million; (2) Haemoglobinuria, the urine contains also red cells and bile pigment; (3) Bloody diarrhoea; (4) A mortality of about 50 per cent. On the other hand the symptoms may be exceedingly slight.

Blood Examination.—Fairly large intracorpuseular and extracorpuseular forms occur.

Post-mortem.—The tissues are very oedematous. The spleen is enlarged. The liver especially, and the kidneys shew marked inflammatory changes. The gut is inflamed and ulcerated.

Transmission.—(1) By inoculation; (2) *Eu. bursa* is the carrier in Hungary. As in *P. canis*, it is only the adult tick, the daughter of an infected mother, that conveys the disease. The incubation period is about seven days. It is uncertain whether the blood of recovered sheep is infective.

Eu. bursa

Adults *take* infection from a sick sheep. Drop on to the ground and lay eggs which develop into

Larvae ; attack a sheep (first host). Moults on sheep in seven to eight days and become Nymphs. After growing (twenty-one days) they fall off and moults on the ground and become Adults. The Adults attack a sheep (second host) and give infection.

8. *P. muris*.—Found in the blood of three albino rats (*Mus rattus*). About one per cent. of corpuscles infected. Typical 'pears' occur. The rats in all cases died from (?) *Piroplasma*.

9. *P. rossi*.—In monkeys, East Africa.

10. *P. sp.*—In sheep. India. Resembles *P. parvum*.

11. *P. cervi*.—In the fallow deer (*Cervus dama*) in Lisbon. Bacillary and cross forms were present, but no typical pears.

12. *P. aristotelis*.—In young hinds (*Cervus aristotelis*) in Annam. Ovoid and cross forms occur. Bacillary forms not found. It may be identical with the previous species.

13. *P. quadrigeminum*.—In *Ctenodactylus gundi*, the gundi, a rat-like rodent of N. Africa. The following forms occur in naturally infected animals :—

(1) Young forms 1μ in diameter. These may become elongated or comma-shaped.

(2) Forms 2μ in diameter having a vesicular nucleus with a large curved chromatin mass and a small punctiform chromatin mass, as in Leishman-Donovan bodies.

(3) Typical pyriform parasites larger than the last. In *Ct. gundi* artificially infected, parasites appear about the third day and in about a week multiplication forms. The parasite divides into four, the segments remaining for some time attached in the form of a fan.

14. *P. vesperuginis* (vide p. 249).

Chapter XXVII

TICKS

Ticks or Ixodidae belong to the zoological group of the Arthropods, as among other characters they have *jointed* extremities; and they belong to the class Arachnoidea which is characterised by possessing *four* pairs of legs. In this class occur also spiders, mites, etc. They thus differ from the insects (Insecta) which have only three pairs of legs. They are divided into two sub-families, the Ixodinae and the Argasinae.

LIFE HISTORY OF TICKS

IXODINAE

Take a neglected dog or other animal infested with ticks, *e.g.* *Eurhipicephalus*, place it in a cage entirely covered with muslin.* Next morning remove from the inside females which have left the dog in order to lay eggs, and larvae and nymphs in order to moult. Place in well-plugged test tubes and observe the following stages.

Egg.—The Ixodinae lay several thousand eggs, the process lasting a week or so. The eggs are laid on the earth or by some species by preference in cracks. During the process the head is forcibly flexed on the belly so that the tip of the hypostome touches the vulva. A membrane now prolapses from the opening of the cephalic gland and protrudes so as to cover the

* Ticks may be fed on cattle, etc., by placing sleeves on the legs or bags over the ears.

head. The membrane is distended and bathed with the secretion from the cephalic gland. The ovipositor now places an egg on the edge of this membrane, which is then withdrawn, the egg being lifted on to the back of the head; the tick moves slightly backward and a pile of eggs is thus deposited in front.

The average size of an egg is 300 to 400 μ .

The female has now become shrivelled, and characteristic bright yellow spots appear under the skin due to accumulation of uric acid in the malpighian tubes and much of this material (white) is passed per rectum. Egg laying takes several days; the tick then dies. The eggs hatch in some weeks or months, and there emerges the

Larva.—These are hexapod (Fig. 94). They cling to blades of grass, etc., and it may be several months before they have the chance of reaching a host. Observe the alimentary sac with lateral and posterior series of diverticula and the rhythmical contractions in these. In the gorged larva note under the microscope the diverticula swollen with blood and the large polygonal cells standing out clearly against the crimson contents. After feeding they go through a dormant pupa-like stage. This whole period lasts from a few days to several weeks. The first moult then takes place, either on the host or after leaving the host, on the ground. In this respect differences occur in different genera and species. Then emerges the

Nymph.—These are octopod (Fig. 94). They resemble adult females, but have no sexual opening. This stage lasts from a few days to several weeks, the nymph becomes engorged with blood and, after the dormant stage, the second moulting takes place, either on the host, or after leaving the host, on the ground. Then emerges the



Unfed larva = active



gorged larva = inactive
(pupa-like)



unfed nymph = active



gorged nymph = inactive
(pupa-like)



unfed adult = active



gorged adult

Fig. 94. Stages of an *Eurhhipicephalus*. $\times 2$

Adult.—After a few days on the host, copulation commences. The mode is not exactly known. It is believed that the rostrum of the male (without the palpi) is inserted into the vulva of the female and by some means or other, or possibly by the help of the palpi, the spermatophores of the male are conveyed to the vulva of the female. In some ticks it is the male that seeks out the females, while in other species the females fight for the male.

When the females are gorged and have left their host (in some species after a day's stay only) they become in a few days less plump and a characteristic yellow mottling appears, due to distension of the malpighian tubes with their secretion. Egg laying is now proceeding. When complete the tick shrivels up and dies. The life of a tick is a variable period. From the beginning of the larval stage to the beginning of egg laying is, for *Eu. appendiculatus*, 11 weeks, for *M. decoloratus*, three weeks.

ARGASINAE

(a) Examine the dust of the floor of rest houses, the gravel in native passenger sheds (in India), the débris beneath 'halting' trees, bed platforms, the hearth in native huts, cracks in walls, etc., etc., for *Ornithodoros*. A sieve is useful for sifting the dust. Confine the ticks in Petri dishes with some gravel.

(b) Place the dust to be examined near a good fire and capture with a moistened brush or finger tip the very young ticks now revealed by their activity.

Egg:—

O. moubata.—Lays eggs at intervals of a week or so, 10 to 20 or even 50 to 100 in a batch. The eggs are the colour of glue, 0.88 by 0.77 mm. Examine with a low

power and note the polygonal reticulation in fresh specimens due to the yolk globules. Note that later the cuticle ruptures and the white embryonic layer and rudimentary malpighian tubules, etc., are visible.

O. savignyi.—Egg-laying lasts about a week, in India, the eggs being shiny black in colour, ovoid, about one mm. long. In about a week emerges the

Larva:—

O. moubata.—The larva does not emerge from the egg. It, however, does so in the Transvaal form of this species (Newstead).

O. savignyi.—The larvae emerge from the egg in about a week. They do not seek blood but remain quietly at rest.

Nymph:—

O. moubata.—Emerges from the egg in one to two weeks. It moults at least three times.

O. savignyi.—The nymph develops from the larva in about a week and is very active, but after feeding becomes quite motionless, resembling a pupa (dormant stage).

Adult:—

These ticks are mainly nocturnal in their habits, and *O. moubata* and *O. savignyi* feed on man principally, if not exclusively, and bury themselves in dry earth, gravel, etc., during the day, and very readily feign death, folding their legs tightly together (*O. moubata*). During feeding, a secretion is poured out of the coxal glands, so that the tick is sometimes bathed in fluid. A bite may casue considerable ecchymosis. After feeding the skin may be moulted, and the tick then becomes very active and is ready to feed again. The male fertilizes several females, and the same female is fertilized by several males (*O. savignyi*). The rostrum has not been seen by us in the vulva of the female.

While the Ixodinae only moult twice during their life, viz., at the change from larva to nymph, and again from nymph to adult ; the Argasinae moult not only at these periods but also several times during the nymphal stage and again several times during the adult stage. The Argasinae also do not become distended with blood to the enormous extent so characteristic of the Ixodinae. The duration of life is probably a year or more.

EXTERNAL ANATOMY

IXODINAE

The Rostrum or Capitulum.—The rostrum consists of a massive posterior portion (head) prolonged anteriorly into the mouth parts.

The dorsal surface of the head is continuous, anteriorly, with two tubular sheaths (mandibular sheaths) within which the rod-like mandibles (or Cheliceres) play (Fig. 95). Ventrally, the head is continued forwards as a conspicuous dagger-shaped

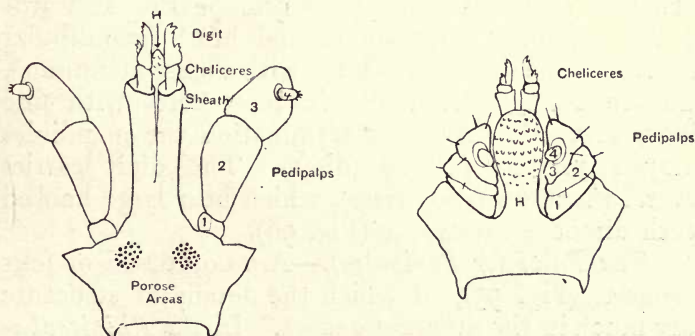


Fig. 95. (Left figure). Mouth parts of *Hyalomma* (an Ixodine).
(Right figure) Mouth parts of an *Eurhipicephalus* (a Rhipicephaline)
H = hypostome ; 1-4 = segments of palpi

process, the hypostome. On either side of the hypostome are the jointed palps or pedipalpi. The hypostome, mandibles and the palps lie, as a rule, closely approximated. In the genera in which the palpi are long (Fig. 95), they are often found separated from the rest of the mouth parts; where the palpi are very short and broad, as in *Eurhipicephalus*, this separation is rarely seen.

The Hypostome (Labium or Radula).—The hypostome (Fig. 95) is continuous with the chitinous exoskeleton of the head. It is bilaterally symmetrical and carries a number of conspicuous teeth, directed backwards, and usually arranged in several rows. The number of longitudinal rows of teeth is very constant in the same species and is used for identification.

The Mandibles (or Cheliceres).—Are strongly chitinated organs (Fig. 95), the anterior portions only of which are seen on external examination. The posterior portions, which are swollen, lie in the body cavity, where they receive the attachment of powerful muscles. The anterior portions are rod-like and play each in a sheath, formed by a prolongation forwards of the chitinous covering of the head (mandibular sheath). The sheaths are lined with a loose membrane, and are covered externally in most cases with fine ridges or teeth. At their termination the mandibles carry a jointed process (digit). The digit carries several processes (apophyses), which bear large hooked teeth directed backwards (Fig. 96).

The Palpi (or Pedipalpi).—Are composed of four segments (Fig. 95), of which the details of structure vary much in the different genera. In the *Rhipicephalinae* the whole palp is very short, thick, and massive. In the *Ixodinae* it is longer, and as a rule, much simpler, in arrangement. In the palps of *Eurhipicephalus* and

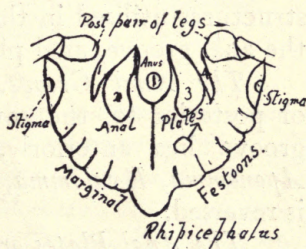
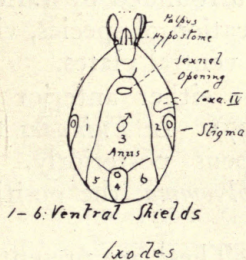
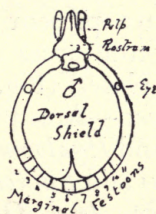
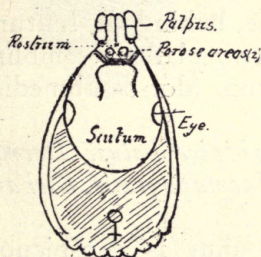
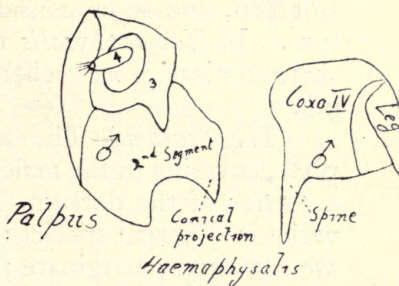
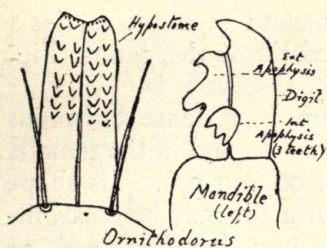


Fig. 96. Illustrating external anatomy of Ticks
(adapted from Neumann)

Haemaphysalis the second and third segments are much enlarged, and show considerable elaboration of structure. In *Haemaphysalis* the second segment carries a sharp conical process characteristic of the genus (Fig. 96).

The Scutum.—The scutum is a dense chitinous plate, covering in the male the *whole*, and in the female, a *portion* of the dorsum (Figs. 96 and 97). Its shape varies in different species; in front it may be emarginate or non-emarginate; it may be furrowed or ornamented with punctures or coloured spots, and perforations (eyes) may be present or absent.

On the dorsum of the female, behind the scutum, there are present, sometimes, two plates resembling the porose areas on the rostrum (dorso-submedian porose plates) (Fig. 97).

Eyes.—Are present in *Eurhipicephalus*, *Derma-centor* and *Hyalomma*, absent in *Haemaphysalis*, *Eschatocephalus* and *Euixodes*.

The Anal Opening.—The anus is conspicuous (Figs. 96, 97, 98). It is situated about one-third of the body length from the posterior margin. The actual opening is slit-like, guarded by two lateral semi-circular plates of chitin. It is surrounded by various structures utilised in the identification of species, viz., the anal groove, anal plates, and ventral plates.

The Anal Groove.—May be either anterior to, or posterior to, the anus (Fig. 97). In *Euixodes* the groove lies anteriorly, and opens posteriorly. In *Aponomma*, *Hyalomma*, and *Amblyomma*, the position is reversed.

The Anal Plates or Clypei.—These are present in the males of certain species (Figs. 96 and 98). They are conspicuous plates of chitin, lying on the ventral surface upon either side of the anus. Four plates,

two on each side, are present in the male of *Eurhipicephalus*. The shape of the plates varies in different species.

The Ventral Shields.—These are, as a rule, less conspicuous sclerites, which cover the ventral surface in the male. The arrangement of these plates, or sclerites, differs in the different species (Fig. 96).

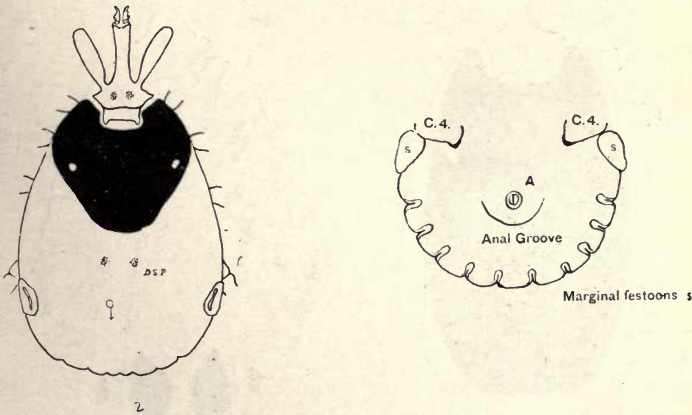


Fig. 97. (Left figure) Female tick (*Hyalomma*). Scutum = black, the white spots are the eyes. D.S.P. = dorso sub-median porose areas.

(Right figure) Posterior ventral extremity of a tick (*Aponomma*);

A = anus; C. 4 = coxa of fourth leg; S = stigma

Marginal Festoons.—Also most marked in the males of certain species, notably in *Amblyomma* (Fig. 96), where they are eleven in number. The median festoon in some males (*Eu. decoloratus*, *M. annulatus*, *v. caudatus*) forms a short but distinct tail (Fig. 96, bottom right-hand corner).

The Genital Opening.—Close behind the rostrum, on the ventral surface, is the opening of the genital

canal (Figs. 96 and 98). It is smaller, and much less conspicuous, than in the *Argasinae*, and in the gorged female is seen only as a minute pore.

The Stigmata.—The stigmal plates or peritremes (Figs. 96 and 98), into which the tracheal system opens, lie behind Coxae IV in the *Ixodinae*; between Coxae III and IV in the *Argasinae*. Considerable variation

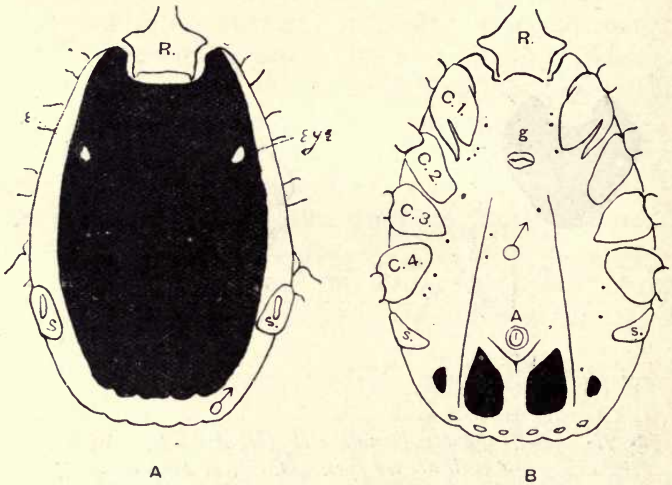


Fig. 98. (A) Dorsal surface of male tick (*Hyalomma*)

R. = rostrum ; e = eye ; s = stigmatic plate

(B) Ventral surface of male tick (*Hyalomma*)

R = rostrum ; C₁-C₄ = coxae ; s = stigma

A = anus ; g = genital opening

Scutum and anal plates = black

exists in the size and shape of these organs in the different species and genera. They also differ in many cases in the two sexes.

The Legs.—The legs consist of six segments, viz., coxa, trochanter, femur, tibia, protarsus and tarsus. The basal segments, *i.e.*, the coxae, are enlarged and

exhibit variations in different species. Coxae I may show large and conspicuous teeth, or may be bidentate (Fig. 99). Coxae IV, in *Haemaphysalis*, carries a spine or tubercle (Fig. 96). Tubercles may be present on all the coxae. The terminal segment (tarsus) may carry one or more 'spurs,' which are of use in identification of species. The tarsus also carries two large curved claws, and a pulvillus (membranous sucker) in the *Ixodinae* but not in the *Argasinae*.

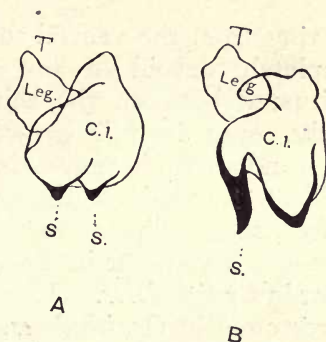


Fig. 99. (A) Front leg of *Eurhipicephalus*
(B) Front leg of *Hyalomma*
T = trochanter ; C.I = coxa ; S = spine

TO DISTINGUISH THE SEXES IN THE IXODINAE

(1) The scutum in the male covers practically the whole dorsum whilst in the female it covers only the anterior third (Figs. 96 and 97) ; as a result the male has often a more uniformly shiny look. The male also is sometimes smaller and more elongate in outline than the female.

(2) The males of certain species possess structures not seen in the female, notably the anal plates and, in some cases, a rudimentary tail (Fig. 96). Marginal

festoons are more marked in males, and the male often has tarsal spurs when they are absent in the female.

(3) The females, on the other hand, may have porose areas, structures not found in the larva, nymph, or male (Figs. 95 and 96). The females alone become gorged in the manner so characteristic of the *Ixodinae*.

ARGASINAE

The head rises from the ventral surface, and the animal is completely devoid of any scutum. The stigmata are situated between the third and fourth legs. The limbs, even in fully-grown and gorged animals, are large and strong in proportion to the body.

The pulvilli of the tarsi are absent in the adult. The palpi are free, short, filiform, and consist of four segments. The eyes are absent in *Argas*, and may be present or absent in *Ornithodoros*. There is a hood-like fold (the camerostome) in *Ornithodoros*, lying in front of and around the base of the head (Fig. 100). This is not present in *Argas*. The abdomen does not become hypertrophied during feeding, as in most of the *Ixodinae*, out of all proportion to the head and limbs, and growth takes place uniformly; the fully developed animals being identical, in all except size, with the smallest forms.

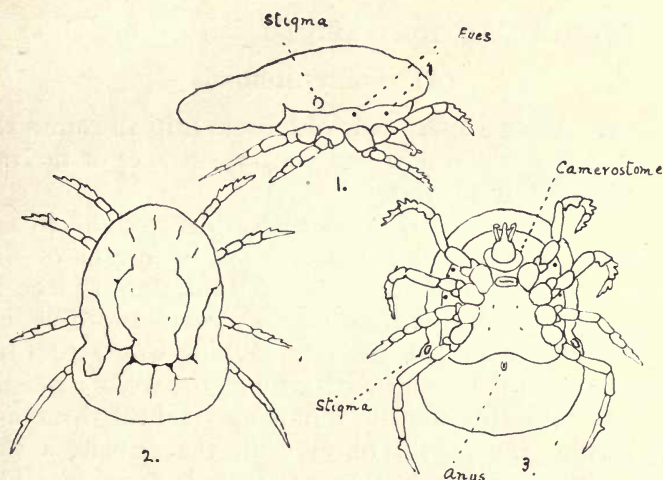
DISTINCTION OF SEXES

The males are with difficulty distinguishable from the females. In the male the genital opening is narrow and semilunar, nearly as long as broad. In the female the opening is an elongated slit with parallel lips, about as broad as the rostrum.

(1) *Ornithodoros*.—Note the deep sulci (Fig. 100), especially

1. The supra-coxal groove, which gives rise to the supra-coxal fold and extends quite around the anterior portion of the body.

2. The pre-anal sulcus, lying just in front of the anus and very conspicuous in sections.



Ornithodoros 1. Side view 2. Dorsal view 3. Ventral view

Fig. 100. *Ornithodoros*: (1) side view, (2) dorsal view,
(3) ventral view

3. The sulci about the head. The conical head can be retracted to a considerable extent into the fold of the integument (camerostome).

At the base of this fold is a groove, and in it the opening of the cephalic gland. This opening can be seen by forcibly flexing the head on to the belly.

On the coxae of the first pair of legs, lying within the groove which separates this from the coxa of the

second pair, there is a pore, from which the secretion of the large coxal gland, described later, is at times exuded.

(2) *Argas*.—Note the pit-like structures on the dorsum and also the absence of the fold around the base of the rostrum.

INTERNAL ANATOMY AND DISSECTION OF TICKS

(A) ORNITHODOROS

1. With a sharp pair of scissors snip all round the edge so that the dorsal and ventral surfaces of the tick are completely separated.

2. Place the tick in a dissecting trough* in salt solution, and fix the ventral chitin by means of fine pins or hedgehog quills. Remove the dorsal piece by grasping it behind with a pair of forceps and dragging it forwards over the head, detaching any structures which may adhere to it with a touch or two of a needle.

3. Whilst carefully removing tracheal tissue and separating the organs observe, in the female, a thin sheet of tissue stretched over the whole dorsum. This is composed of fat tissue and tracheal branches.

Heart.—In the central line is the tubular heart dilated, at about the junction of the posterior with the middle third of the body to form a conspicuous sac.

Alimentary Diverticula.—Note that there is a central short median ventricle or sac receiving anteriorly the oesophagus and ending posteriorly in the fine, almost capillary, tube which passes to the rectum. Trace out a number of anterior, lateral and posterior blind

* A dissecting trough can be purchased, or readily made in the following way. Put some pieces of lead at the bottom of a convenient dish and pour over them a mixture of melted paraffin wax (melting point about 60° C.) and soot or fine animal charcoal.

diverticula which arise from the median sac. Ascertain that the posterior and some of the lateral diverticula are of extreme length, and pass round to the ventral surface, reaching to the neighbourhood of the anus, vulva, and even the rostrum (Fig. 101).

Some of the lining cells of the diverticula are especially large, and project freely into the lumen. These and other smaller cells become, during digestion, loaded with jet black granules.

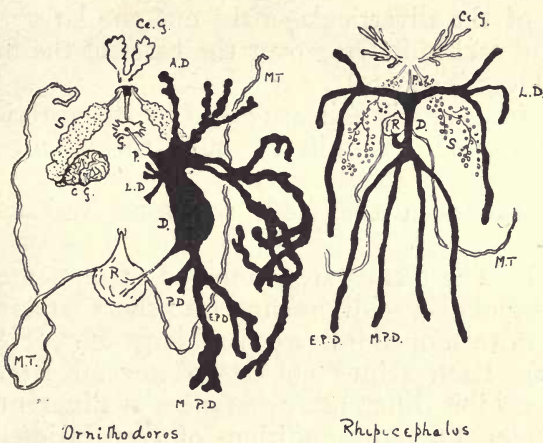


Fig. 101. Alimentary canal and appendages of *Ornithodoros* and *Eurhipicephalus* (semi-diagrammatic)

C.G. = coxal gland ; Ce.G. = cephalic gland ; D = ventriculum ;
 A.D. = anterior diverticulum ; L.D. = lateral diverticulum ;
 P.D. = posterior diverticulum ; M.P.D. = median posterior
 diverticulum ; E.P.D. = external posterior diverticulum ;
 P = proventriculus ; R = rectum ; S = salivary gland ;
 M.T. = malpighian tubule

Some time after feeding, in the still partially fluid blood, a number of reddish granules are seen with the naked eye : they are really cells, wandering digestive

cells of doubtful origin, containing a large number of yellowish or black granules of large size.

A peculiar phenomenon takes place in the wall of the sac as digestion proceeds; the inner layer escapes through the meshes of the outer in the form of a number of herniae, so that the whole gut appears as if studded with cysts; a condition very marked in *Ornithodoros*, but not to the same extent in *Eurhipicephalus*.

Salivary Glands.—By displacing to one side the whole of the diverticula, make out the large salivary gland of each side lying over the bases of the first and second legs (Fig. 101).

Lifting up the salivary gland by its posterior end, trace forward the salivary duct till it enters the chitinous ring of the pharynx.

Examine in the fresh condition and ascertain that—

(1) The glands are composed of immense numbers of globular acini opening into short lateral ducts, which in turn open into a central large duct (Fig. 102).

(2) Each acinus consists of several large cells, which exhibit different appearances at different stages and under different conditions of preparation. Note the globules of secretion packing these cells when mature.

(3) In sections note a large clear cell at the point of entrance of the duct, and the different stages in the formation of the large secretory granules.

Coxal Glands.—Lying partly under the salivary gland and partly internal to this structure observe a large flask-shaped organ, the coxal gland, conspicuous from the number of tracheae which supply it (Fig. 101).

Cephalic Glands.—By displacing the diverticula from the extreme anterior portion of the body make

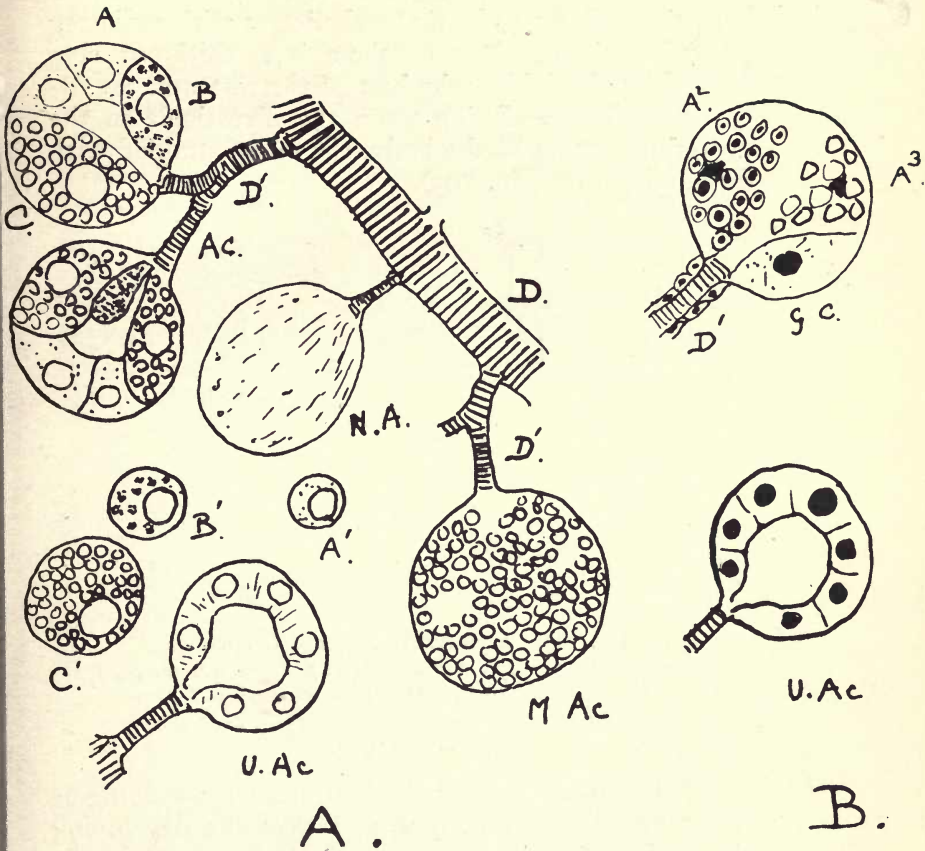


Fig. 102. Structure of Salivary Gland. (A) fresh; (B) stained. D = main duct; D¹ = duct; Ac = acinus; A = finely granular cells not yet possessing secretion; B = coarse granules which become changed into secretion; C = globules of secretion; A¹, B¹, C¹ = the same cells set free; M.Ac = mature acinus filled with secretion; U.Ac = undeveloped acinus; N.A = remains of nymphal acinus; G.C = Granular cell; A², A³ = granules of secretion.

out the bilobed cephalic gland (Fig. 101), and further back the bulbous ends of the cheliceres with radiating muscular fibres. Around these is a chitinous ring formed by the fold at the base of the rostrum.

The Ovary.—Lying upon the diverticula in the posterior portion of the body, the ovary studded with developing ova (Fig. 103).

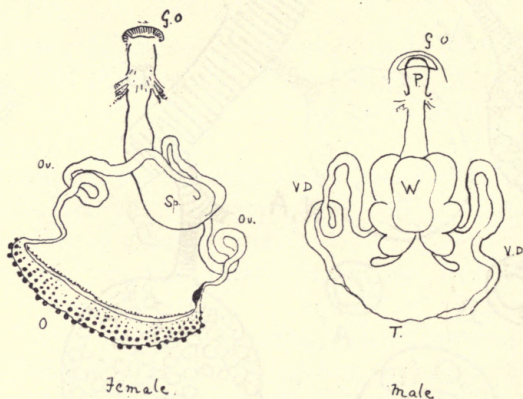


Fig. 103. Reproductive organs of *Ornithodoros*
 Female: O = ovary; Ov = oviduct; Sp = spermatheca;
 G.O = genital orifice. Male: T = testis; V.D =
 vas deferens; W = white gland; P = penis;
 G.O = genital orifice

Note that there is a single ovarian sac opening at either end into an oviduct, and that the developing ova project from the outer wall of the sac and only pass into the cavity at maturation (Fig. 104).

By aid of sections and fresh preparations observe that

(a) Each ovum is attached to the ovary by a funicle, composed of a single tube of modified ovarian cells. When the cavity of the ovary is distended this tube is obliterated and the cells form a thick plate.

(b) A portion of the ovary consists of a delicate, much wrinkled membrane, which does not bear ova.

Spermatheca.—Upon either side of the ovary the coiled oviducts ending in the middle line in the large bilobed spermatheca (Fig. 103). Open the spermatheca, and note the presence of cyst-like bodies (spermatophores) containing masses of spermatozoa.

Rectum.—Behind the spermatheca, observe the filamentous termination of the alimentary canal in the dilated rectum (Fig. 101).

Note the thin walls, the entry of the two malpighian tubes into it, and its opening at the anus. Note the oval or the dumb-bell shaped crystals of the contained secretion.

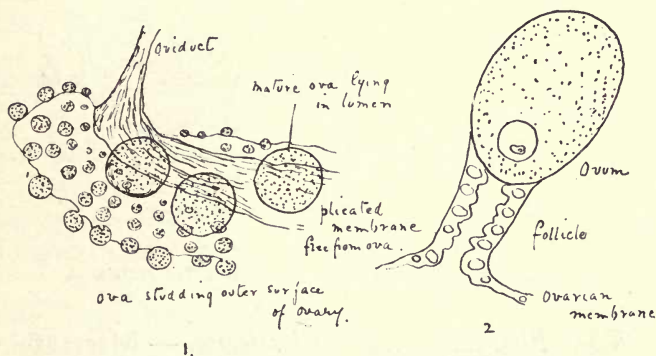


Fig. 104. (1) Portion of ovary of *Ornithodoros*;
(2) Ovum and follicle

Malpighian Tubules.—Note the great length of these and their tortuous course among the viscera (Fig. 101). Trace them from their origin in the rectum to their blind termination in the anterior portion of the body. Note, in some cases, small dilatations containing secretion similar to that in the rectum.

Male Reproductive Organs.—Observe a similar arrangement to the female organs, the testis being, however, a smaller and finer tube than the ovary and ending on either side in coiled vasa deferentia, which finally enter the large ‘white gland.’ The ejaculatory duct ends in a chitinous penis (Fig. 103).

The Mouth Parts.—Behind the place of the origin of the palps a small portion of the mouth is completely closed in. Here the pharynx opens ventrally by a slit-like opening protected by a narrow tongue of chitin; and into the two posterior angles open the large salivary ducts of either side (Fig. 105).

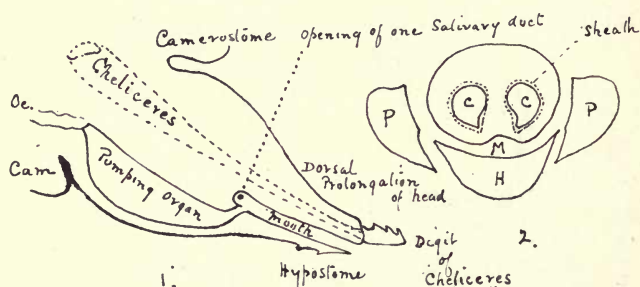


Fig. 105. Mouth-parts of *Ornithodoros*
(1) Longitudinal section; (2) Transverse section

The Pharynx and Endosclerite.—Macerate a specimen of *Ornithodoros* after removing the dorsal chitin by boiling in caustic potash (ten per cent.). Make out the following (Fig. 105):—

(1) A broad sheet of chitin which lies horizontally and divides the cavity of the rostrum into an upper and a lower portion. This projects behind well into the body cavity, and is connected anteriorly with the dense chitin about the base of the palps and back portion of the buccal cavity.

(2) The two fang-like cheliceres lying loosely each in a sheath *above* the horizontal plate and nearly filling the upper cavity of the rostrum.

(3) A triradiate pumping organ (pharynx) lying *below* the horizontal plate and occupying, with its muscles, the lower cavity of the rostrum.

(B) EURHIPICEPHALUS

The Gorged Female.—Obtain a large gravid female and remove the dorsal chitin, as in *Ornithodoros*, but avoid cutting through the rostrum. Observe that, in the main, the organs are similar to those of *Ornithodoros*, but that the following differences are present (Fig. 101):—

(a) The system of diverticula is simpler, and there are no anterior branches.

(b) The ovary is much longer and thinner, and the spermatheca relatively much smaller.

(c) The salivary acini are much more loosely packed so that the organ, as a whole, is less easily differentiated.

(d) The cephalic gland consists of a number of long finger-like projections.

(e) There are large dermal glands lying among the fat and tracheal tissue.

The Ungorged Female.—Place on a slide and with a sharp knife bisect the tick along the middle line. Place one of the halves in a drop of saline, and fixing the extreme outer edge by means of a needle, scoop out the whole of the contents with a scalpel, disturbing the arrangement of the tissues as little as possible. Under a lens most of the viscera are readily isolated and can be removed for study to another drop of saline.

The Male.—Treat as in the case of the ungorged female. Note the distended *vasa deferentia* and the narrow alimentary diverticula (Fig. 101).

The Nymph.—To prepare specimens of the freshly gorged nymph cut off, by pressing with a sharp scalpel, a thin slice from the posterior end, and passing the scalpel from before backwards, press out the whole of the contents into a drop of normal saline. Search for separate organs with a low power lens. To obtain specimens of the tissues of nymphs which have advanced towards metamorphosis, treat as in the case of the ungorged female, or better, cut off the anterior one-third of the body by means of a sharp scalpel and turn out its contents. Observe that a rough model of the future adult in embryonic tissue is present.

For observing developmental stages of *Piroplasma*, make smear preparations of this tissue and look under a low power for parts where salivary ducts and young acini are forming.

EXAMINATION OF TICKS

Place a tick alive upon a slide and cover with a second slide. Pass a rubber band over each end (or tie with thread) so as to compress the tick into an extended position suitable for examination. (Rubber bands are easily made from a piece of rubber tubing).

In this way note the characteristics of (*a*) the unfed larva; (*b*) the gorged larva; (*c*) the unfed nymph; (*d*) the gorged nymph; (*e*) the unfed female adult; (*f*) the unfed male adult; (*g*) the gorged female adult (Fig. 94).

To Mount Ticks—

Method I. (*a*) Place the ticks mounted as above

(slides, rubber bands and all) into absolute alcohol for about an hour.

(b) Remove the rubber bands and place the tick in (CuSO₄) absolute alcohol for about another hour.

Clear in oil of cloves and lower into a large drop of thickish balsam so as to avoid air bubbles.

Method II. Kill by placing in boiling water, then proceed as in Methods I, III, etc. The tick is suitably extended by this method.

Method III. (a) Boil the compressed tick for from ten to thirty minutes, or even much longer, in ten per cent. caustic alkali, until it looks quite transparent.

In the case of soft bodied ticks, especially gorged nymphs and larvae, it is necessary to regulate the pressure, or they may become ruptured either before or after placing them in the solution. For nymphs and larvae coverglasses may be used in place of slides.

(b) Remove the tick and wash thoroughly in water till quite free from alkali.

(c) Stain in a saturated solution of fuchsin (if desired), wash in alcohol.

(d) Dehydrate, clear and mount in balsam.

Method IV. For ticks that are already dry, boil in ten per cent. potash or soda till clear. Wash in water, dehydrate in alcohol, clear in oil of cloves and mount in balsam. (Stain in fuchsin after the boiling, if necessary).

It is often advisable to open the tick or remove some of the dorsal integument in order to see the mouth parts clearly (Newstead).

Method V. *To Cut Sections of Ticks.*—Take ticks, as young as possible, best of all those that have only just moulted, then proceed as on page 116.

Method VI. *To Examine Single Organs.*—Dissect out the organs, *e.g.*, the salivary glands. (1) Smear a portion out on a slide, fix, and stain, or (2) fix the whole organ in a suitable fixative (*vide* p. 404), imbed in paraffin.

Preservation of Ticks.—This may be done in the following ways : (1) Dry ; (2) ten per cent. formalin ; (3) spirit.

Despatch of Living Ticks by Post.—Ticks will travel alive in pill boxes with the lid replaced by some fine gauze, or place in specimen tubes with some pieces of crumpled paper to prevent shaking. Plug the tube with wool.

Chapter XXVIII

The Ixodidae are classified in the following way* :—

- | | | | |
|---|---|--|--|
| I | { | Sub-family <i>Ixodinae</i> .—(a) Scutum present ; (b) pulvilli present on claws ; (c) the mouth parts project and are seen in front ; (d) stigmata behind Coxa IV - 2 | |
| | | Sub-family <i>Argasinae</i> .—(a) Scutum absent ; (b) pulvilli absent on claws of adults ; (c) mouth parts hidden on the under surface of body, so that they are not seen from above ; (d) stigmata between Coxae III and IV - - - - - 9 | |
| 2 | { | Section <i>Ixodeae</i> .—(a) ♂ has ventral side of body covered with shields or plates ; (b) anal groove surrounds the anus in front and is not connected with the genital grooves ; (c) eyes absent ; (d) rostrum long - - - - - 3 | |
| | | Section <i>Rhipicephaleae</i> .—(a) ♂ has two adanal plates, often with accessory plates ; (b) anal groove surrounds the anus behind and joins the genital grooves in front ; (c) eyes present ; (d) rostrum long or short - 5 | |
| 3 | { | Section <i>Amblyommeae</i> .—(a) ♂ ventral plates absent ; (b) anal groove surrounds the anus behind and joins the genital grooves in front ; (c) eyes often present ; (d) rostrum long or short - - - - - 7 | |
| | | Second and third segments of palpi grooved on inner surface in ♂ (p. 318) - <i>Euxodes</i> (= <i>Ixodes</i>) | |
| | | Palpi not grooved in ♂ - - - - - 4 | |

* The method of using this and similar tables is as follows. (1) Determine if a tick has or has not certain characters contrasted in group 1. If the tick has, e.g., pulvilli then proceed to group 2, if not, to group 9. If it is in group 2 then determine again whether or no it has various characters, e.g., if it has no eyes proceed to group 3, if it has eyes to 5 or 7, and so on, until the genus is reached.

4	{	Third segment of palpi not long, etc., legs very long (p. 320) -	<i>Eschatocephalus</i> (= <i>Haemalastor</i>)	
		Legs not very long. Third segment of palp, long acuminate, extending beyond point of articulation of fourth (p. 320) - - - - -	<i>Ceratixodes</i>	
5	{	Rostrum long (p. 326) - - - - -	<i>Hyalomma</i>	
		Rostrum short - - - - -	- - - - -	6
6	{	Stigmata comma shaped (p. 321)		
		<i>Eurhipicephalus</i> (= <i>Rhipicephalus</i>)		
7	{	Stigmata round (p. 323) <i>Margaropus</i> (= <i>Boophilus</i>)		
		Rostrum long - - - - -	- - - - -	8
8	{	Rostrum short - - - - -	- - - - -	9
		Eyes present (p. 325) - - - - -	<i>Amblyomma</i>	
9	{	Eyes absent (p. 328) - - - - -	<i>Aponomma</i>	
		Second segment of palp has a prominent lateral projection giving the palp a triangular or crescentic appearance (p. 324) - - - - -	<i>Haemaphysalis</i>	
10	{	Second segment of palp has not a lateral projection (b) Coxa IV, very large and characteristic in the male (p. 328) - - - - -	<i>Dermacentor</i>	
		Body flat, with thin edges, finely shagreened (punctate) narrow in front (p. 330) - - - - -	<i>Argas</i>	
		Body, thick sides, covered with warts (p. 328)	<i>Ornithodoros</i>	

GENUS EUIXODES

(= *Ixodes* [*sensu strictiori*]).—Eyes absent. Palpi long; hollowed on the internal surface in both sexes. Tarsi without terminal spurs. Anal groove surrounds anus anteriorly and opens posteriorly. Scutum in male does not cover the body laterally and posteriorly. Stigmata oval in ♂, circular in ♀. Male ventrally covered with six shields; two lateral, embracing the origins of the legs and the stigmata; one median,

between the genital opening and the anus ; two on each side of the anus (perianal) ; and one triangular posterior shield, carrying the anal orifice at its anterior corner. Female has dorsally three longitudinal grooves on the abdomen, ventrally, two bell-shaped grooves, the first has its apex at the vulva, the second at the anus.

(1) *Evi ricinus* (= *reduvius*).—The castor-bean tick. ♂. Sides of anal shield divergent (*i.e.*, the anal shield is 'open'). Scutum covered with very fine hairs (pubescence), covers the greater part of back, and is not fringed with hairs behind. Coxa I with a strong spine internally only. Tarsi not knobbed. Ventrally, the rostrum has a little knob just behind the base of the palp.

♀. Anal grooves prolonged behind the anus, divergent. First segment of palpi short, not forming a horn directed forwards. Scutum longer than broad, with rounded or angular sides (not concave), has cervical and lateral furrows. Coxa I unicuspid, tarsi not knobbed. This tick is common on sheep, goats, and cattle, Europe.

Pathogenicity.—Transmits *P. bigeminum*.

It is probable that *Evi ricinus* takes infection (*P. bigeminum*) in adult stage and gives in larval and nymphal stage.

Bionomics :—

1. Egg laying -	100-1,000 eggs laid	Time 1-2 weeks
	Incubation -	- „ 6 „
2. Larva -	(a) On animals -	„ 1 „
	(b) On the earth. At	
	the end of this period	
	the first moult takes	
	place and then results	„ 4 „

3. Nymph - (a) On an animal - Time 1 weeks
 (b) On earth second
 moult takes place - „ 8 „
4. Adult - On host ; copulation
 takes place - - „ 1 „

These periods vary with circumstances. The tick can fast for long periods at any stage, and can hibernate also at any stage.

(2) *Evi hexagonus* is the European dog tick.

GENUS CERATIXODES

(= a sub-genus of *Ixodes*).—Palpi long : in ♂ inner surface not grooved so as to embrace the mouth parts. In ♂, palpi (third segment) end in a conical spine (*i.e.*, acuminate), the fourth segment being inserted almost at the base of the former. In ♀, third segment slightly grooved and the end slightly swollen. No eyes. No anal groove in ♀. In ♂ one anal shield, two adanal. Stigmata circular in both sexes.

C. putus.—Found on sea-birds, and crawling about the cliffs where they breed.

GENUS ESCHATOCEPHALUS

= (*Haemalastor*) a sub-genus of *Ixodes*.—Eyes wanting. Rostrum long. Palpi piriform and not grooved in ♂, claviform or flat in ♀. Anal grooves as in *Ixodes*. Stigmata circular in both sexes. Legs very long. Dorsal and ventral chitinous thickenings in the male ; fine grooves in the female. They occur on bats and in caves.

GENUS EURHIPICEPHALUS

(= *Rhipicephalus* in part).—Eyes present. Base of rostrum when looked at from above hexagonal forming an angle on each side. Differ from genus *Margaropus* in the following points:—

- (1) Stigmata comma shaped in ♂, shorter in ♀.
- (2) Eleven marginal festoons.
- (3) Anal groove present in ♀.
- (4) First segment of palp bears on the ventral inner surface a plate which may be prolonged into a hook or spine and which bears five to seven feather bristles (cp. *Margaropus*).

(5) Coxa I deeply incised, producing two long cusps.

♂. A single pair of anal plates, the accessory pair only slightly developed; in *Margaropus*, they form a second pair.

♀. Stigmata round. Scutum, polygonal, posteriorly; not triangular as in *Margaropus*.

Bionomics.—*Eu. evertsi* changes its host between the nymphal and adult stage, while *Eu. appendiculatus*, the 'brown tick' and *Eu. simus*, the 'black-pitted tick,' change their host after the larval and nymphal stage.

(1) *Eu. appendiculatus*.—♂. Palpi flat dorsally, with convex margins. Tooth on first segment well developed. Scutum uniform in colour. Marginal furrows single. The posterior accessory furrows join the festoons. Eyes flat, scutum with numerous, unequal, chiefly fine, generally regularly scattered punctations. 'Tail' twice as long as broad (only visible when the male has fed for some days). Coxa I with a long anterior prolongation visible from the dorsum (cp. *Eu. sanguineus*).

♀. Palpi flat dorsally, with convex margin; tooth on first segment well developed. Scutum: brown, eyes flat, belly uniform colour. Scutum as broad as long, or a short oval; anal margin not fringed with white. Scutum: with distinct, somewhat unequal, fine punctations. Marginal furrow clearly marked. Porose areas as a rule separated by *twice* their diameter.

Pathogenicity.—Transmits *P. parvum* (p. 283).

(2) *Eu. simus*.—♂. Marginal furrow single, the median and accessory furrows in front of the hind margin very slightly, if at all, indicated (characteristic). Marginal festoons are long, and are separated by deep furrows. Adanal shields not pointed. Ext. spiniform 'shield' present. Eyes flat, large, and yellow. Scutum brilliant reddish brown; punctations large and equal, arranged more or less in four rows with or without additional fine, hardly visible, punctations.

♂. Eyes flat, scutum broader than long, punctations distinct, unequal, the fine ones little visible. Porose areas separated by a distance equal to their diameters. Lateral angle of the rostrum not conspicuous. It is the 'black-pitted tick' of S. Africa.

Pathogenicity.—Transmits *P. parvum*.

(3) *Eu. evertsi*.—♂. Shield shagreened; uniform deep brown colour with red border. Marginal furrow simple. Eyes spherical. Characteristic are the legs, saffron red. Eleven marginal festoons unbordered with white as in *Eu. capensis*.

♀. Shield shagreened. Eyes brilliant and spherical. Legs red. The 'red-leg tick' of S. Africa.

Pathogenicity.—Transmits *P. equi*. Larvae and nymphs which have fed on infected animals transmit in the adult stage (p. 286).

Bionomics.—Passes the first moult on, the second moult off the host. It attacks the regions around the anus.

(4) *Eu. bursa*.—♂. Scutum reddish brown, with equal, fine, distant, punctations. Marginal furrow present, simple. A postero-median and accessory furrows present. Characteristic are the triangular anal plates with broad hind margin and accessory spine external to them. Length 4 mm.

♀. Scutum dark reddish brown, oval, nearly as broad as long. Often concave behind the eyes. Punctuation numerous, thick, equal. Anal margin is not white. Belly uniform in colour. Europe, Africa, West Indies, Malay. It occurs on the goat in India.

Pathogenicity.—Transmits *P. ovis* in Europe.

Bionomics.—(Vide p. 290).

(5) *Eu. sanguineus*.—♂. Eyes flat. Scutum : marginal furrow single, punctations clearly seen, regular, unequal, numerous. Characteristic are the two roundish shagreened pits on each side of the postero-median (dorsal) furrow. These do not reach the posterior margin. Tail short or absent, not so prominent as in *Eu. appendiculatus*.

♀. Scutum hollowed out in front, a long oval, the angle being rounded. Punctations of unequal sizes. Porose areas separated, as a rule, by about their diameter. In both sexes Coxa I has the inner process long and sharp, not rather stumpy as in other species. Found on dogs in most tropical and sub-tropical countries.

Pathogenicity.—Transmits *P. canis*.

GENUS MARGAROPUS (= BOOPHILUS)

Differs from *Eurhipicephalus* in the following points :—

- (1) Stigmata round ; (2) marginal festoons absent ;
- (3) anal groove absent ; (4) palpi : second and third

segments thicker in the middle forming a sharp angle externally. First segment has not five or seven bristles on its ventral inner chitinous plate as *Eurhipicephalus* has; (5) Coxa I only slightly incised, with two stumpy processes. ♂ very small, about two millimetres. Scutum very convex, punctate, four rows of hairs. Two pairs of anal plates having their sides parallel, not triangular. External plate as long as internal.

♀. Very small scutum, posteriorly it is V-shaped, not polygonal as in *Eurhipicephalus*.

Bionomics.—Ticks of this genus remain on their host all their life, from the larval stage till they drop off to lay eggs. From egg to adult stage lasts about three weeks and the whole life is about nine weeks.

Neumann considers that there is only one species, viz., *M. annulatus* (= *bovis*), and that *australis decoloratus*, *caudatus*, etc., are merely varieties. Dönitz considers that there are at least two species.

(1) *M. decoloratus*.—The blue tick. ♂. The four anal plates all end in a sharp point, often projecting beyond the hind margin. First segment of palpi on the lower and inner side has a stumpy appendage bearing a single bristle. Tail present. Radula (hypostome), six rows of teeth. Rhodesia, Cape Colony.

Pathogenicity.—Transmits *P. bigeminum*.

(2) *M. annulatus* (= *Boophilus bovis* and *M. australis*).—The common blue tick. Tail absent. Radula, eight rows of teeth. Japanese varieties have ten rows of teeth. Anal plates have stumpy ends.

Pathogenicity.—Transmits *P. bigeminum*.

GENUS HAEMAPHYSALIS

Base of rostrum rectangular, twice as broad as long. Eyes absent. Anal shields absent in ♂. Second

segment of palpi has a well-marked lateral conical projection. Palp as a whole distinctly triangular or crescentic. Stigmata circular or comma shaped anteriorly. Coxa I not cleft as in *Eurhipicephalus*. Coxa IV not enlarged as in *Eurhipicephalus*, but has a well marked spur.

(1) *H. leachi*.—Second segment of palp has a sharp spine especially well marked in ♂; on the inner side eight bristles; third segment has a strong hook; fourth segment covered with numerous hairs. Hypostome four or five rows of teeth.

♂. Scutum yellowish red, punctations regular and equal. Marginal furrow well marked. Venter white. Hind angles of rostrum sharply pointed. Coxae have short spines; that on Coxa I the largest.

♀. Second palpal segment with sharp external angle. Scutum oval. Punctations regular, cervical grooves much prolonged posteriorly. Marginal grooves absent. Porose areas separated by their diameter. The South African dog tick.

Pathogenicity.—Transmits *P. canis*.

Bionomics.—It leaves its host for each moult.

(2) *H. flava*.—The common goat, sheep, and buffalo tick of S. India.

GENUS AMBLYOMMA

♂. Eyes, generally flat, but not always conspicuous. Anal grooves as in *Aponomma*. Anal plates absent, but there are two pairs and one median chitinous platelet near hind border (Dönitz). Rostrum long, behind almost rectangular, curving forwards to the narrower anterior portion. Palpi long. First segment very small, second long with four or five bristles, fourth in a pit on the underside of the third. Hypostome, three

or four rows of teeth. Scutum often with coloured designs of specific importance. Stigmata usually triangular. Eleven festoons in the male.

(1) *A. hebraeum*.—♂. Marginal festoons all green except the two outside ones which are brown. Scutum: sulphur colour marked with brown, the pattern not in the form of an **H** or **Y**. Median posterior linear spot separate from the anterior spots. Marginal groove extends to the posterior border. Legs brown with white distal spots on segments 2, 3, 4, and 5.

♀. Scutum as broad as long, triangular posterior borders almost straight. On margin of scutum one or two minute 'eye-spots,' *i.e.*, a dark point surrounded by a bright circle. Punctuation scanty, thick on sides. Shews metallic lustre. Eyes spherical, flat in anterior quarter of scutum. Coxa I bicuspid. Coxa IV no spine. S. Africa.

Pathogenicity.—Transmits 'heartwater' in sheep, the cause of which is unknown.

Bionomics.—The tortoise, variegated or bont tick. Leaves its host for each moult. It is a very large species, and the females will only bite if males are already *in situ*.

(2) *A. americanum*.—The Lone Star tick; on cattle.

GENUS HYALOMMA

Eyes present. Rostrum long. Anal groove as in *Aponomma*. Body elongate, oval, colour more or less deep brown.

♂. Very long palpi; two pairs of ventral plates, the inner triangular, the outer smaller and straight. Stigmata comma-shaped with a long tail. Scutum festooned posteriorly. Tarsi spurred.

♀. Stigmata triangular. Not easily distinguished from *Amblyomma*, the following differences, however, occur :—

(a) Rostrum without sharp angles as in *Eurhipicephalus*, as the postero-lateral border is very short, and the antero-lateral slopes gradually into the anterior.

In *Amblyomma*, the antero-lateral and postero-lateral sides of the rostrum form a uniform curve and the antero-lateral curves gradually.

(b) The plate on the ventral surface of the first segment of the palpi is smaller than in *Eurhipicephalus*, and bears a number of closely-packed bristles. In *Amblyomma* it is reduced to a mere ridge bearing only one or two bristles.

(c) The eyes are situated about the middle of the sides of the scutum : in *Amblyomma* generally in front of this point by half or one-third of the length.

(1) *H. aegyptium*.—The speckled leg, or bont (motley) leg tick. A very fine and large tick. Scutum : punctation large, unequal and very irregular in distribution, may be black or brown. Legs uniform or bont (motley). Plate of the first palpal segment elongated antero-posteriorly, has seven bristles.

♂. Coxa I deeply divided, adanal shields with hinder part of median border longer than the posterior border. The median marginal festoon is small, triangular, and often white.

♀. Eyes brilliant. Scutum as broad or broader than long, numerous unequal punctations; deeply indented behind the eyes. Coxa I deeply divided. Africa, S. India, Asia, Europe.

Bionomics.—Larvae and nymphs have not been found on cattle; they possibly infest birds. It is common on the camel and horse.

GENUS APONOMMA

Eyes wanting. Anal groove surrounds anus posteriorly, and opens anteriorly. Anal plates absent. Base of rostrum pentagonal. Scutum covers the dorsum entirely; usually marked with green spots. Stigmata, comma-shaped. Female, scutum shorter than broad, three green spots. The species are parasitic on reptiles.

GENUS DERMACENTOR

Coxa IV very large. Eyes present. Base of rostrum broader than long. Palpi short and thick. Stigmata, comma-shaped. Anal shields absent in male. Coxa I bidentate in ♂ and ♀. Scutum ornamented. About twenty-four species.

(1) *D. electus* is the American dog tick.

(2) *D. occidentalis* conveys 'spotted fever' of the Rocky mountains, the cause of this disease being, however, unknown.

GENUS ORNITHODOROS

Eyes present or absent. Rostrum hidden under a projecting beak (head) close to the margin of the body so that the tips of the palpi only are visible from above. Body generally oval, sides generally straight, skin mammillated throughout. Lateral margin not different as in *Argas*. No moveable plate on each side of palpi.

(1) *O. savignyi*.—Has two pairs of eyes situate on a horse-shoe shaped elevation surrounding the base of the legs and the mouth parts. Hind tarsi are slenderer than in *O. moubata*. On the last segment of the hind legs are three knobs, the distance between the second and third three times as great as that

between first and second. Skin covered with hemispherical warts. Attacks man. Africa and India.

(2) *O. pavimentosus*.—Eyes present. Body covered with flat warts above forming a pavement. Distance between the knobs on last segment of hind legs as in *O. savignyi*. Habits similar to those of *O. moubata*. S. Africa.

(3) *O. morbillosus*.—Eyes present. Has only two knobs on hind tarsi. Africa.

(4) *O. moubata* (= *O. savignyi*, var., *caeca*).—No eyes. Distance between the first and second and second and third knobs on last segment of hind legs about equal. This segment is stouter and more compressed than in the three previous species. Africa.

Pathogenicity.—Transmits *Sp. duttoni*, probably in nymphal stage mainly.

Bionomics.—In *O. moubata* the larva does not leave the egg, but moults inside and leaves it as the eight-legged nymph.

It is a night feeder, leaving its host after it has sucked enough blood, to conceal itself during the day time. Eggs are laid and the ticks moult after each blood meal. Their life extends to a year or so, whereas in the Ixodidae it is only some months.

(5) *O. megnini*.—The spinose ear tick in America. Attacks animals and man, occurring mainly in the ears.

(6) *O. turicata*.—Attacks pigs and man.

GENUS ALECTEROBIUS (Pocock)

Differs from *Ornithodoros* in having a fold of skin capable of being folded under the palp.

(1) *A. talaje*, the 'chinche' of S. America and elsewhere, is very troublesome to man.

GENUS ARGAS

Eyes absent. Rostrum, which is concealed by cephalo-thorax, is situated at least its own length behind the anterior margin. No projecting head. Body oval or elliptical, flat or hollow dorsally, with fine granulations. The edges with a lateral ridge with different markings from those on rest of dorsal surface. No groove behind the anus.

(1) *A. miniatus*.—Body oval, margin formed of rectangular festoons. Dorsal submarginal granulations contiguous. The American fowl tick.

Pathogenicity.—Transmits *Sp. gallinarum* in S. America.

(2) *A. persicus*.—For its injurious effects famous to travellers from Persia to Peking. A poultry pest in S. Africa.

GENUS CARIS

Body almost as wide as long. Has a conspicuous transverse groove behind the anus, differing from *Argas* in this respect.

(1) *C. vespertilionis*.—Parasitic on bats.

LITERATURE

(a) NEUMANN'S Monographs are indispensable for the identification of ticks.

Mem. Soc. Zool. de France, t. IX, 1896, pp. 1-44; t. X, 1897, pp. 324-420; t. XII, 1899, pp. 107-294; t. XIV, 1901, pp. 249-372; the last containing tables of all the genera and species.

Archives de Parasitologie, 1902-1907.

Annals of Tropical Medicine and Parasitology, Vol. I, No. I, 1907.

(b) SALMON and STILES. Cattle Ticks of the United States, XVIIth Annual Report, 1900, Department of Agriculture. An excellent summary, costing a few shillings only.

(c) DÖNITZ, W. Die wirtschaftlich wichtigen Zecken mit besonderer Berücksichtigung Afrikas.

Chapter XXIX

THE TRYPANOSOMIDAE

The *Trypanosomidae* comprise two genera—

(1) *Trypanosoma*, (2) *Trypanoplasma*.

Trypanosoma.—The genus is characterised by the possession of a longitudinal undulating membrane, the thickened border of which takes its origin posteriorly from a blepharoplast, and terminates anteriorly in a free flagellum. Division takes place longitudinally.

Structure of Trypanosomes.—In the gut of the louse certain *non-flagellate* forms of *T. lewisi* are described, which have a single oval nucleus only. From this nucleus eventually arises by division the blepharoplast, or nucleus regulating motility. This shews that this structure is nuclear in origin and, moreover, as the flagellum now appears at the end of the young developing trypanosomes where the blepharoplast is, this flagellum-bearing end is the anterior one; and it is only later that it takes up its ordinary posterior position. Besides the blepharoplast, the undulating membrane, and the flagellum, myonemes are also described. These ectoplasmic fibres are not generally seen in the ordinary methods of staining. They are most readily demonstrated in large trypanosomes like *T. rotatorium* or *T. theileri*, especially if the trypanosomes have been eviscerated by the pressure of making a blood film or by the action of macerating fluids.

Besides these details of minute structure, some authors consider that trypanosomes exhibit—

Sexual Differences.—These differences are not

generally easily appreciable, at least in the blood, but as we shall see later, such forms have also been described in the gut of flies transmitting particular trypanosomes, and the differences are there said to be well marked. In the case of *T. brucei*, for example, three types are described in the blood.

(1) *Male Forms*.—Excessively slender forms staining deep blue, with a sharply defined, rather long chromatin-rich nucleus. They are more actively motile than other forms.

(2) *Female Forms*.—Flagellum short. Membrane slender, little folded; two or three times as broad as other forms. They stain a light blue; have few or no granules and the nucleus is spherical.

(3) *Indifferent Forms*.—The most numerous form. The nucleus is not sharply defined, and the protoplasm contains numerous granules.

It must, however, be remembered that all transitions appear to occur between these forms, and that observers are by no means agreed as to which of these forms are which; and finally it must be noted that the amount of granules and the depth of staining of the protoplasm of a trypanosome can be made to change at will by varying the composition of the Romanowsky and by washing for variable lengths of time.

Mode of Division.—This is by longitudinal division. The trypanosome at the time of division increases considerably in thickness. The nucleus and blepharoplast divide, sometimes one first, sometimes the other. A new flagellum is formed by an out-growth from the new blepharoplast; or according to others by a splitting of the base of the old flagellum. Finally the protoplasm also divides. For rosette formation *vide* under *T. lewisi*.

Encysted Forms.—In animals treated with atoxyl

at a time when trypanosomes are decreasing in the blood, the majority of trypanosomes are disintegrated into a mass of débris, but some become rounded and encysted (Moore and Breinl).

Latent Forms.—During the course of an infection when trypanosomes are *decreasing* in the blood, Moore, and Breinl describe forms found at first in the lungs and somewhat later in the spleen and bone marrow (and in small numbers in the blood), which they call latent forms. They are minute forms consisting of a nucleus with an intranuclear body (centrosome) and a vesicle, the whole lying in a thin film of protoplasm. These exist, then, in the organs (a few also in the blood)



Fig. 106

when trypanosomes are absent from the blood, and some of these give rise again to young, and then full-grown trypanosomes which multiply by longitudinal division as described above. Trypanosomes thus appear to have, at least in the case of a rat infected with *T. gambiense*, a regular cycle in the body. It is possible that these minute forms are the source of infection in blood which, although filtered, is still infective.

Involution Forms.—These are the amoeboid forms of some observers. They have lost their flagellum and are found under various unfavourable conditions, *e.g.*, in the blood of animals under treatment with atoxyl, etc., also in the spleen (Fig. 106), or in blood that has been heated to 40° for one hour, or in *post-mortem* blood.

DISTINCTION OF SPECIES

A difficulty that confronts everyone who is dealing with an unknown trypanosome is the question of its identity with species already known. There are many ways of attempting to solve this question.

1. *Morphology*.—Though it is frequently difficult to distinguish pathogenic trypanosomes, *e.g.*, *T. brucei* and *T. evansi*, yet some can be readily distinguished; *e.g.*, *T. equinum* of Mal de Caderas can be easily distinguished by its extremely minute blepharoplast, and similar differences exist in varying degrees among morphologically similar trypanosomes.

In making such comparisons the trypanosomes must always be taken from the same kind of animals *e.g.*, guinea-pig.

2. *Pathogenicity*.—*T. theileri*, apart from its morphological characters, is distinguished by the fact that it is specific for cattle, *i.e.*, it cannot be inoculated into other animals; forming with *T. lewisi* (specific for rats) Koch's two specific trypanosomes. On the other hand most pathogenic trypanosomes (non-specific, Koch) can be inoculated into a variety of animals. A comparison of the results got by these inoculations is used as a means of establishing the identity of two trypanosomes, *e.g.*, the trypanosomes of human trypanosomiasis and sleeping sickness have identical effects on animals, and hence are judged to be identical. Again, *T. equiperdum*, though closely resembling *T. brucei* is easily distinguished from it by the fact that cattle are refractory to it. This method, however, has its drawbacks; as two trypanosomes, almost certainly identical, coming from the same locality and from among the same herd of animals may differ so considerably in virulence, that

one would be inclined to consider them quite distinct did one not know their origin.

3. *Immunity*.—The best test of the difference between two trypanosomes lies, perhaps, in the fact that an animal immunized against one trypanosome will yet succumb to another, *e.g.*, Laveran and Mesnil base their belief in the non-identity of Ngana and Surra on the following fact. A goat or cow recovered from *T. brucei* infection is inoculated again with *T. brucei*. No effect is produced, and its blood in several c.c. is not infective for other animals. It is now inoculated with *T. evansi*, and its blood becomes infective even in a few drops. It cannot here be a question of difference of virulence of different strains, for *T. brucei* is as virulent as *T. evansi* for cattle. Again, mice infected with *T. equinum* and cured by Trypan-red or arsenic are susceptible to *T. equiperdum*, and when cured of *T. equiperdum* mice are susceptible to *T. brucei*.

4. *Precipitin Test*.—Mayer added to the serum of a dog infected with *T. brucei* the salt extract of centrifugalised *T. brucei* trypanosomes. A copious precipitate resulted. With the serum of a dog infected with *T. equinum*, he got no result.

AGGLOMERATION OF TRYPANOSOMES

Under various conditions trypanosomes come together and form rosettes, sometimes of about a hundred individuals. In these the posterior ends are central, united by a slimy secretion derived from the blepharoplasts (?), and the flagella are peripheral. They may be seen in the peritoneum after injection by this path. Chemical reagents, immune sera, and also normal sera, are in particular cases capable of producing

the effect. In these rosettes there is no loss of motility, and the clusters may separate again. In *cultures*, e.g., of *T. lewisi*, the trypanosomes are agglomerated by their *anterior* ends (Fig. 107).

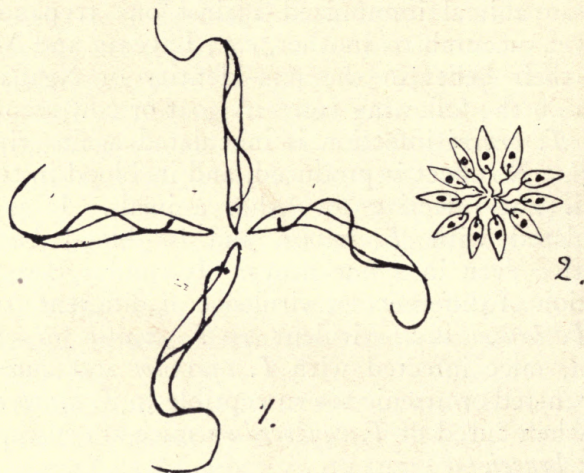


Fig. 107. (1) Agglomeration of trypanosomes; (2) Culture forms (*Herpetomonas*) in rosette

CULTIVATION OF TRYPANOSOMES

Novy-MacNeal-Mathis Medium.—1. Collect the blood of any suitable animal, e.g., cow, rabbit, etc. (strict asepsis is not absolutely necessary).

2. Defibrinate by shaking up with wire, or glass beads, etc.

3. Add one part of blood to two of agar in a number of tubes.

4. Sterilize once or twice at 100°C., and make slopes. (*Vide* also p. 353.)

5. Inoculate the condensation water with a little blood of the trypanosome to be grown, e.g., *T. lewisi*, and keep at room temperature.

6. Examine from day to day, and make sub-cultures when a good growth is got (in about a week).

Trypanosomes in cultures are generally small, 5 to 15 μ , but may be much larger, and they nearly always are of the *Herpetomonas* (p. 362) form.

1. *T. gambiense*.—The cause of sleeping sickness or better human trypanosomiasis, a disease of tropical Africa. It is stated that native dogs (in Uganda) also harbour the parasite.

Incubation Period.—Is not easy of determination, but can last months and probably years.

Symptoms.—(1) A general polyadenitis, *i.e.*, enlargement of the lymphatic glands, especially the cervical. (They may in some cases decrease again in size.)

(2) Fever of an irregular remittent character, but frequently at first taking the form of an evening rise followed by a fall in the morning.

(3) Acceleration of the pulse and rate of breathing.

(4) Skin lesions; patches of erythema, *e.g.*, on the thorax, with congestion and some oedema giving a purplish appearance. A papulo-vesicular eruption is also common, especially in the native.

(5) Oedema; especially of the face, giving a puffy look, also on the legs and elsewhere.

(6) Nervous symptoms; first perceived, perhaps, as a mere alteration of expression or disposition. These eventually show themselves in headache, unsteady gait, tongue tremors, tremor of the outstretched hand, commencing apathy, disposition to sleep, lethargy, coma, death. Occasionally 'sleep symptoms' are entirely absent.

1. *Blood Examination*.—Parasites may be absent from the peripheral blood for a month or more at a time, and even if abundant, seventy to a cover-slip, may again completely disappear. The number of

parasites bears no relation to the severity of the symptoms.

If necessary, dilute the blood (several c.c.) with one per cent. sodium citrate solution, and centrifugalize; carefully remove with a pipette some of the superficial layers of the sediment, and examine with a one-sixth inch lens fresh. If not found, the fluid is poured off, some fresh citrate added, and the process repeated three or four times.*

2. *Blood Changes.*—In uncomplicated cases of sleeping sickness there is no anaemia (?). As regards the leucocytes the changes are not constant. A large mononuclear increase up to twenty to thirty per cent. has been recorded, and even higher values for native blood. On the other hand the increase generally affects the lymphocytes, which may reach values of fifty to sixty per cent. It is probable that some of these are plasma cells, but they have not been so far recorded. The possibility of a mononuclear increase from trypanosomiasis should therefore be borne in mind in those cases of malaria where no parasites are present, but trypanosomiasis in doubtful cases is most easily diagnosed by gland puncture. The average values of a large number of counts made by Greig and Gray give: red cells, 4,707,000; white cells, 11,000; large mononuclear, twelve per cent.; lymphocytes, thirty-eight per cent.; polynuclears, thirty-nine per cent.; eosinophil, five per cent.

3. *Gland Puncture.*—Sterilize the syringe and get rid, as far as possible, of any fluid in the interior. Puncture the posterior cervical glands and move the point of the needle about in the substance of the gland. Draw out the piston. Now detach *the barrel only*. Next take out the needle. Then, replacing the barrel eject the contents, best, into a

* Koch recommends the frequent examination of stained films made as thick as possible.

minute drop of citrate solution or salt (Greig and Gray). Examine with a one-sixth inch. This is by far the easiest and most certain way of making a diagnosis.

Lumbar Puncture.—Place the patient on his right side with the knees drawn up to the face, so as to get a position of extreme flexion of the lumbar vertebrae. The tips of the fingers of the left hand are then placed upon the left iliac crest, when the thumb will indicate the site of puncture (between the fourth and fifth lumbar vertebrae) which lies half an inch to the left of the middle of a line joining the two iliac crests. Insert a stout hypodermic needle for one to two inches until it is felt free in the canal. Draw off about twenty c.c. and centrifugalise for half an hour. Examine the sediment. Within one hundred days of death trypanosomes are practically always present, except sometimes a day or so before death.

5. *Scarification.*—Of the erythematous patches often seen in trypanosomiasis in the European, may give a positive result when the blood examination is negative.

Occurrence of Streptococci.—A diplo-streptococcus can frequently be isolated by culture from the glands and cerebro-spinal fluid in cases of sleeping sickness. Thus Greig and Gray, in an examination of eighteen cases which terminated fatally, found in the glands streptococci in fifty per cent., and trypanosomes in one hundred per cent.; in the cerebro-spinal fluid streptococci in forty-four per cent., and trypanosomes in one hundred per cent. of cases; but in the majority of cases the streptococci were detected a day or so, or even only a few hours before death, so that the streptococci represent a terminal infection and are not in any way the cause of the onset of symptoms. Further, death can occur with all the typical symptoms and yet the organs prove sterile.

Morphology.—17-28 by $1.5-3\mu$. The blepharoplast is oval. There is not uncommonly a vacuole in close association with it. The trypanosome, at least in animals, occurs in two main forms, a long and a short. With regard to the alleged sexual forms occurring in the fly *vide* p. 341.

The Spleen.—Is considerably enlarged and congested.

Brain.—The pia-arachnoid shews patchy thickening and opacity, especially at the base. Microscopically the brain shews an absolutely characteristic change, viz., chronic inflammation of the perivascular lymphatics. Around the vessel there is always present a neuroglia cell proliferation, and the branches of these cells form a reticulum. In this reticulum occur a large number of lymphocytes and plasma cells of Marschalko (p. 8).

Besides this characteristic cell there are found also makrophages, which are possibly altered perivascular lymphatic endothelial cells; so-called morular or granule cells, *i.e.*, cells containing a number of spherules that stain with eosin; and degenerated trypanosomes and chromatin particles, probably nuclear remains of the former.

Lymphatic Glands.—Are soft and moveable, and the deeper ones may be as big as a walnut. They shew increased vascularity (haemolymph glands) and chronic inflammatory changes resembling those in the brain, *viz.* :—

(1) Lymphocytes in all stages up to plasma cells occur.

(2) Proliferation of the connective tissue cells of the reticulum of a lymph sinus, and marked proliferation of the nuclei of the endothelial cells.

(3) When the inflammation subsides they become fibrous.

(4) The glands have often suppurating points due to streptococcus infection.

*Transmission.**—From an infected to a healthy individual is effected by *Gl. palpalis*, and almost certainly by other species, though these have not as yet been accurately determined. The transmission is direct, *i.e.*, the fly passes straight from the sick to the healthy person; in fact, according to experimental evidence on animals, the fly cannot infect a second animal, as the adherent trypanosomes are cleared off in the skin of the first animal bitten. Further, the fly that has bitten an infected animal ceases to be infective on the next day, and probably much earlier (? a few hours). In spite of this fact, the trypanosomes are said to undergo the following changes in the mid-gut of the fly, (1) In twenty-four hours two kinds of trypanosomes appear, (a) the female, with sluggish motion, large with granular and deeply staining cytoplasm; the nucleus is large and spherical, the free flagellum is short, and the blepharoplast is some way from the posterior end (Fig. 108). These are rare in blood films. (b) The male actively motile and slender, cytoplasm non-granular, nucleus usually compressed, free flagellum long. These are common in blood films. (2) In forty-eight hours trypanosomes of an indifferent type appear. These are the type that prevail in blood, and have a short free flagellum. (3) In ninety-six hours all trypanosomes have disappeared, nor can they be found in any other of the flies' tissues. If development now proceeds in some unknown way, then the fly must become infective again later. It does not appear that experiments have been made with flies kept for long periods.

A certain percentage, one to seven percent., of tsetse flies, moreover, that have never fed on human blood and possibly not on blood at all, contain trypanosomes in

* Also occurs through sexual intercourse in some cases (Koch).

their gut. These are of two kinds, possibly more. (1) *T. grayi* ♂ very slender and very long compressed nucleus; blepharoplast anterior, free flagellum long (Fig. 108). ♀ bulky, protoplasm containing many staining granules (chromidia), nucleus oval or round, blepharoplast variable in situation, free flagellum very short. Young and indifferent forms, many of the

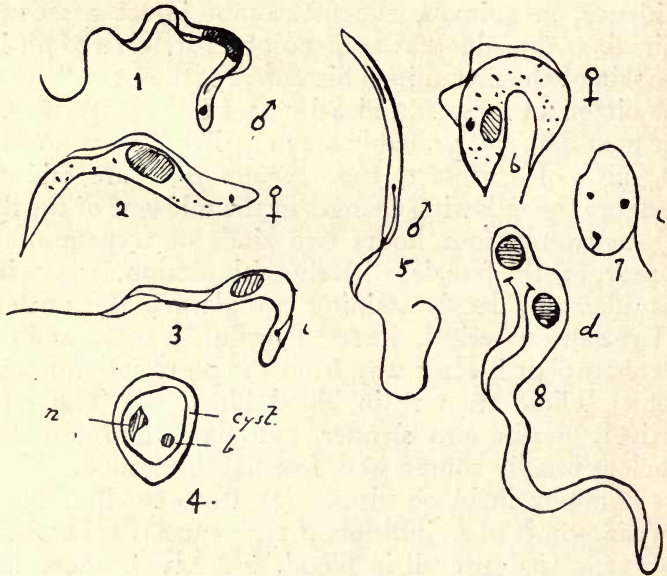


Fig. 108 (1) *T. gambiense*, ♂; (2) *T. gambiense*, ♀; (3) *T. gambiense* (indifferent form); (4) *T. grayi*, encysted stage; (5) *T. grayi*, ♂; (6) ♀; (7) indifferent; (8) dividing form

latter being round and also containing chromidia. Dividing forms are characterized by the fact that one is much larger than the other; the smaller having the blepharoplast anterior to the nucleus, while the larger has it posterior.

Encystation of T. grayi.—In the hind gut of one glossina Minchin found trypanosomes becoming encysted. The flagellum was withdrawn and absorbed, and the body then became surrounded by a definite cyst.

With regard to the further development of a trypanosome taken into the stomach of a fly, several cycles are possible :—

(1) It may develop in the fly and pass out through the proboscis as the malaria parasite does—this Minchin calls the inoculative cycle.

(2) It may become encysted in the gut and pass out in the faeces, to be swallowed by another host. From the gut of this second host it may pass into the blood stream. This is the contaminative cycle. To what extent this occurs with any trypanosome, is at present unknown.

(2) *T. tullochi*.—It resembles *T. gambiense*. The nucleus is rounded near the middle of the body, the blepharoplast is circular.

These trypanosomes, natural to the fly, can be distinguished from *T. gambiense* (i) by their far greater activity, (ii) by their morphology, (iii) by adding a little goat's serum; the fly trypanosomes rapidly become immobile, whereas *T. gambiense* is uninfluenced.

According to Koch, *T. gambiense* can occur in the salivary glands of Glossinae.

2. *T. brucei*.—The cause of Ngana. This fatal disease or its varieties is widely spread throughout Africa. It occurs in horses, cattle, mules, and many other animals, excepting man. (The ' Jinja ' cattle trypanosome of Uganda is probably the same as this).

Symptoms.—(1) Remittent fever, (2) oedema variable in extent and sometimes fugitive in character, frequently affecting the belly and genitalia. (3)

Marked anaemia and progressive emaciation. (4) Opacities in the eye, leading to blindness. The symptoms are most typical in the horse. Death occurs in a few weeks or months, the appetite being retained to the end. In cattle the disease is less typical; and in other animals, *e.g.*, goats, the symptoms are slight, the course chronic, and recovery may take place.

Incubation.—Is generally about ten days after the bite of the fly.

Blood Examination.—Parasites generally first appear with the onset of a rise of temperature, and they are most easily found during a rise in the temperature curve. The number is very variable, and if not found the oedematous areas should be examined. If the result is still negative, inject five to ten c.c. of blood into a rat, intraperitoneally.

Morphology.— $26-27\mu$ in rats. $28-33\mu$ in horses. The nucleus lies almost in the middle. The blepharoplast is almost quite round. The flagellum is generally separated from it by a slight interspace. (*Vide* also under *T. evansi*.)

Pathology.—There is generally great enlargement of the spleen, liver and lymphatic glands.

The spleen contains much haemosiderin, the reticulum of the pulp is hyperplastic and contains cells resembling myelocytes. The subcutaneous tissue is oedematous, and effusions occur into the serous cavities, *e.g.*, pericardium. The serous membranes may shew ecchymoses. Parasites are found in the exudates, and in large numbers in the bone-marrow. In the spleen they are few, but on the contrary many degeneration forms occur here. In the rabbit there is a round cell infiltration of the testes, and an almost complete degeneration of the seminal tubules.

Transmission.—This is effected by *Gl. morsitans*,

principally, but also by *Gl. palpalis* (?) and *Gl. pallidipes* (?).

The fly, after biting, remains infective from twelve to forty-eight hours, but not longer. It is possible, however, that it may become infective again much later. It has been known since Livingstone's time that horses and cattle become diseased after passing through a 'fly-belt.' The flies transmit the trypanosomes which exist in the wild game. Trypanosomes have been found in wildebeest, kudu, bushbuck, etc.; the wild game, though infected, are apparently healthy. Cattle and other animals suffering from a chronic form of the disease also give a constant supply.

Cultivation.—Is difficult at first, but sub-cultures grow well and are fully virulent.

3. *T. evansi*.—The cause of Surra and its varieties. It is known in India, Burma, Indo-China, Java, the Philippines, Mauritius, and the varieties in N. Africa.

It occurs naturally in horses, mules, camels, etc. Cattle, as a rule, enjoy considerable immunity, yet in the outbreak in Mauritius twenty-five to one hundred per cent. died.

Symptoms.—Are similar to those of Ngana, viz., (1) remittent fever, (2) progressive anaemia and emaciation, (3) oedema, (4) discharge from the nostrils and eyes, (5) muscular weakness and paralysis. Death occurs in some days, weeks, or even months.

Blood Examination.—Parasites are found frequently with difficulty. They are particularly scanty during periods of apyrexia. Make a subinoculation in doubtful cases.

Morphology.—22-30 μ in rats, 35 μ in horses. Laveran and Mesnil, who have made a comparison of *T. brucei* and *T. evansi*, state that *T. brucei* is shorter and more compact than *T. evansi*. The movements of

T. brucei are also less extensive. The posterior end of *T. brucei* is also blunter than that of *T. evansi*. The free portion of the flagellum is shorter in *T. brucei* than *T. evansi*, and the protoplasm of *T. brucei* has more numerous and larger granules than that of *T. evansi*. The nuclei and the blepharoplasts are morphologically indistinguishable. Further, the mean length of *T. brucei* is less than that of *T. evansi*, and the width of *T. brucei* is greater. The distinction between Surra and Ngana is, however, best proved by the fact that an animal immunized against Ngana is yet susceptible to inoculation with Surra.

Pathology.—The spleen is enlarged and there is general glandular enlargement.

Transmission.—Rogers got positive results by means of Tabanidae. *Stomoxys* *sp.* has been suspected, but there are no positive experimental data.

El Debab (= Surra).—A disease of camels in Algeria, is transmitted by *Atylotus nemoralis* and *A. tomentosus*.

Mbori (= Surra).—A disease of Sudan camels, is transmitted by *Tabanus ditaeniatus* and *T. biguttatus*.

Aino.—A trypanosomiasis of camels in Somaliland, is also possibly Surra. Likewise *T. vivax* affecting cattle, sheep and goats in the Cameroons is, according to Laveran and Mesnil, Surra.

Cultivation.—Is even more difficult than in the case of *T. brucei*. Novy and McNeal state that Surra from the Philippines does not form rosettes in culture like *T. brucei*, whereas Laveran and Mesnil with the Mauritius Surra obtained them. It is possible, then, that these also are varieties.

4. *T. equinum*.—The cause of Mal de Caderas, a disease of horses in Central and South America.

Symptoms.—(1) Remittent fever; (2) Progressive

anaemia and weakness; (3) The most characteristic symptom is paralysis of the hind quarters. This first shews itself in a dragging of the hoof, and progresses until the animal can no longer stand; (4) Haemoglobinuria is not uncommon; (5) Oedema is rare; (6) Eye symptoms also occur.

Course.—The disease is nearly always fatal, and lasts a few days, weeks, or months. In donkeys the disease is chronic, lasting six to twelve months. Cattle, goats and pigs are very refractory to inoculation.

Blood Examination.—Trypanosomes are most easily found during the pyretic periods. A sub-inoculation into a dog or rabbit is often necessary for the detection.

Morphology.— $22-24\mu$ by 1.5μ . The main characteristic is the extremely small (? existent) blepharoplast.

Pathology.—The spleen is much enlarged, also the liver and lymphatic glands. The kidneys shew haemorrhagic nephritis. Effusions occur in the various serous cavities.

Transmission.—The mode is unknown. It is possible that ticks are concerned, for the evidence is rather against biting flies.

Zousfana.—Is possibly a variety of *T. equinum*. It affects horses in Algeria. Oedema is nearly always absent, and attacks of haemoglobinuria lasting one to two days are common.

5. *T. equiperdum.*—The cause of Dourine, a disease of horses in Europe, N. America, Algeria (especially), India.

Symptoms.—Three periods are described :—

(1) Period of oedema :—Eleven to twenty days after coitus, swellings of the genitalia appear. There is also fever.

(2) Period of 'Plaques':—In forty to fifty days after coitus well defined areas as big as a shilling or crown piece appear, representing angioneurotic oedematous patches. These plaques last a week or so, or may suddenly appear and again disappear.

(3) Periods of paralysis and extreme anaemia:—Skin abscesses and eye symptoms occur. Paralysis of the hind quarters develops, so that the animal cannot stand. Death occurs in two to ten months or even much later. Ruminants are refractory to inoculation.

Blood Examination.—Trypanosomes are found only with great difficulty, but fairly easily in the area of a plaque if examined at its first appearance.

Morphology.— $25-28\mu$ long. The protoplasm does not as in *T. brucei* contain chromatin granules, and the posterior end is often slightly cleft.

Transmission.—Is effected by coitus of an infected stallion with a mare or *vice versâ*. The resemblance to syphilis should be borne in mind.

Pathology.—The inguinal lymphatics and testes are infiltrated and caseous. According to Mott, following the primary sore there is inguinal gland enlargement, then general gland enlargement. The virus is conveyed along the lymphatics of the pelvic plexus of nerves to the lumbo-sacral ganglia primarily, and to all the spinal ganglia eventually. This leads to the angioneurotic eruption (plaques) which may subside leaving no trace, or it may end in patches of leukoplakia if the inflammation be intense enough to destroy the ganglion cells. Likewise secondary degeneration of the posterior roots, causing an ataxic condition of the hind legs, or a general neuritis may be associated and cause paraplegia.

6. *T. theileri.*—The cause of a disease in South Africa known as gal-ziekte (gall sickness). A similar

if not identical trypanosome occurs in Togo, German East Africa, Transcaucasia and India (*T. lingardi*).

Symptoms.—(1) Progressive anaemia; (2) Slight fever lasting a few days only. The disease in South Africa is often complicated by infection with Piroplasma or Spirochaetes. The trypanosome is strictly specific, *i.e.*, only cattle can be infected.

Blood Examination.—Trypanosomes are found during the first fortnight of the disease.

Morphology.—(1) It is the largest of known trypanosomes. Two forms occur (a) *T. theileri*, (i) large forms $60-70\mu$ by $4-5\mu$; (ii) small forms $25-530\mu$ by $2-3\mu$. (b) *T. transvaaliense*. In this form the oval nucleus is posterior almost touching the blepharoplast $18-50\mu$ by $4-6\mu$. Whether these two forms are different, or varieties, or developmental forms of one another, is doubtful.

(2) Another characteristic feature is the extremely drawn-out posterior end.

(3) The blepharoplast is oval, situated at right angles to the long axis (cp. *T. lewisi*), and often appears as if partly divided (diplosome).

In this trypanosome, from its great size, the myoneme fibres are fairly readily seen, in stained specimens, in those parts where the trypanosomes have suffered compression.

Pathology.—There is icterus of the tissues. The spleen and mesenteric glands are enlarged. Whether these changes are due to the trypanosomes or some other co-existent infection is doubtful.

Transmission.—The disease in South Africa is transmitted by *Hippobosca rufipes*.

7. *T. dimorphum* (Laveran and Mesnil, 1904).—The cause of a chronic disease in horses in Gambia.

This trypanosome is found also in domestic cattle, dogs, pigs, and rarely also in sheep and goats.

Symptoms.—(1) Weakness. (2) Intermittent fever. (3) Oedema of testes, but not elsewhere, as in Ngana. (4) Paraplegia. The disease lasts many months, or over a year, and recovery may occur.

Blood Examination.—Parasites may be scanty at first and more numerous when the febrile attacks are well marked. Later they again become rare, but may be abundant before death.

Morphology.—Three forms occur :—(1) Tadpole form in the early stage of the disease, $11-13\mu$ by $0.18-1\mu$. (2) Long forms, $26-30\mu$ by $1.6-2\mu$ a few days before death. (3) Stumpy forms, $16-19\mu$ by $3.4-3.5\mu$.

According to Lühe, No. (1) represent an indifferent; No. (2) a male(?); No. (3) a female trypanosome.

Transmission.—*Gl. palpalis* is capable of transmitting infection.

8. *T. cazalboui* (Laveran, 1906).—The cause of Souma, a fatal disease of ruminants in French Soudan.

Symptoms.—Oedema, paresis, and cutaneous eruptions urticarial in character. It is non-infective for monkeys, dogs, and rodents, but goats and sheep are readily infected.

Transmission is by *Stomoxys bouffardi*.

9. *T. soudanense*.—The cause of a disease of dromedaries in Sudan, probably a variety of Surra. The pathogenic action on mice is similar to that of the trypanosomes of El Debab and Zousfana.

10. *T. pecaudi*.—The cause of Baleri, a disease of equines in French Soudan. The symptoms are (a) repeated febrile attacks, (b) eye symptoms, (c) skin eruptions and plaques, resembling those of Dourine, (d) oedema of genitals. Two forms occur (1) $25-35$ by 1.5μ , (2) $14-20$ by $3-4\mu$. It resembles *T. dimorphum*.

11. *T. lewisi*.—In a certain percentage of rats all over the world. Trypanosomes may not infrequently be cultivated from the blood of rats in which repeated blood examination has been negative (Novy).

Symptoms.—Non-pathogenic, with very rare exceptions. It can only be inoculated into rats, so is strictly specific. (Cp. *T. theileri*). It is not the same, therefore, as *T. rabinowitschi v. criceti* of the hamster.

Blood Examination.—Rats naturally infected may shew few or very numerous trypanosomes: they may remain infected for months. In inoculated rats, rosettes may be found during the first few days.

Sub-inoculation.—The best method is intraperitoneally, but sub-cutaneous injection suffices. Piebald or old rats may be refractory.

Immunity.—Rats that have become free from trypanosomes are immune. By injecting such rats on several occasions with *T. lewisi*, their serum acquires protective properties.

Morphology.— $7-30\mu$ by $1.5-3\mu$. The posterior end is drawn out into a point. The blepharoplast is rod-like and transverse to the long axis. The nucleus is oval and situated at the junction of middle and anterior third of the body. The protoplasm shews fine chromatin granules, but not coarse ones as the pathogenic trypanosomes do. Division, as in all trypanosomes, is longitudinal, but owing to continued division of the nuclei and delayed division of the protoplasm, rosettes are also formed. These consist of a number of minute trypanosomes with their flagella situated peripherally (Fig. 109). They are characterized by the fact that the blepharoplast has moved close to the nucleus or even anterior to it. The blepharoplast resumes its ordinary position when the rosettes break up.

Transmission.—Infection has been effected by means of a rat flea (*P. fasciatus*) and, it is stated, by the rat louse (*Haematopinus spinulosus*) also. With the louse we have experimented, but always in vain. Prowazek, who, however, also failed to transmit infection by the louse, describes a complex developmental cycle in the gut of the louse.

(1) Fertilisation of a female (thick form, with spherical nucleus) by a male trypanosome (slender, with a slender nucleus) occurs. (2) Formation of an ookinet with a single spherical nucleus. (3) Development of a trypanosome from this ; as the flagellum now

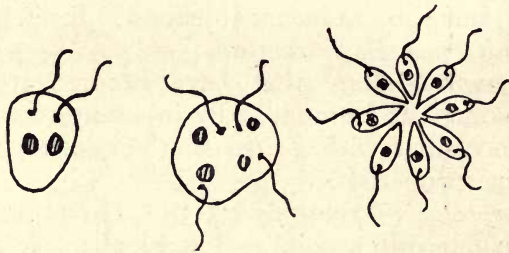


Fig. 109. *T. lewisi*: shewing rosette formation as the result of division

appears at the same end as the blepharoplast, this is taken to be the anterior end of the trypanosome ; later, the blepharoplast moves away to the opposite (posterior) end, viz., that of its normal position. Besides this cycle, the trypanosomes are also said to assume gregarine-like non-flagellate forms, which penetrate between the epithelial cells. How far these forms belong to *T. lewisi* and how far to possible natural trypanosomes of the louse's gut, remains to be seen.

Cultivation.—(Novy and MacNeal). Mix agar, melted and cooled to 50° C., with defibrinated rabbit's

blood, in equal parts, or two parts of blood and one of agar.

The blood may be got by bleeding from the carotid, or by inserting a trocar and cannula directly into the heart, through the thorax, in an anaesthetized rabbit. Collect in a sterile jar, shaking well with pieces of glass rod or wire, etc. Mixing the blood and agar in flasks or as ordinary 'slopes,' and after covering with rubber caps, place in the incubator, at 37° , over night, to make sure of sterility. (*Vide* also p. 336).

Inoculate the tubes with rats' blood containing *T. lewisi*, taken from the heart by means of a sterile pipette, and incubate at 25° C. As soon as a good growth (rosettes) is obtained, sub-cultures should be made.

The cultural forms vary in length from $1-2\mu$ to 60μ . Most characteristic are the rosettes, consisting of large masses of trypanosomes with their flagella centrally placed. The position of the blepharoplast is anterior instead of posterior, and the undulating membrane but little developed. The cultures are infective, and Novy and MacNeal state that, after passing the culture fluid through a Berkefeld filter, the filtrate is still infective.

In Muridae, we have also *T. longocaudense* in the Indian *Mus niveiventer*, and *T. duttoni* in the Senegal mouse *Mus musculus*, and *T. blanchardi* in *Myoxus nitela*, garden dormouse. *T. myoxi* in *M. avellanarius*, red dormouse. *T. bandicotti* (? = *T. lewisi*) in the bandicoot *Nesokia gigantea*.

TRYPANOSOMES IN BATS

(1) *T. nicolleorum* in *Vespertilio kubli* and *Myotis murinus* in Africa. A small (24μ) active form and a large (30μ) sluggish form are described.

(2) *T. vespertilionis* (Battaglia, 1905) in *V. noctula* and other bats in Portugal, 8-12 μ . The motion is peculiar, resembling the bending of mosquito larvae. These and other trypanosomes of bats are at present very imperfectly known.

Other Mammalian Trypanosomes.—*T. nanum* (Laveran, 1905), in cattle in Sudan, with no free flagellum. *T. himalayanum* (Lingard), 75 μ long, in Himalayan cattle. *T. suis* (Ochmann, 1905), in pigs in East Africa; very short and thick, and with very short flagellum. *T. congolense* (Broden, 1904), in sheep in the Congo, 10-15 μ , with no free flagellum. *T. (jinjaense)*, in cattle in Uganda (probably = *T. brucei*), *T. (asino-caballi)* in mules in Uganda.

All these are pathogenic. We have also *T. talpae* in the mole, and *T. cuniculi* (R. Blanchard, 1906) in the rabbit, and *T. pestanai* (Bettencourt and Franca, 1905) in the badger (*Meles taxus*). *T. indicum*, (Lühe, 1906) in the Indian squirrel (*Funambulus palmarum*), *T. spermophili* in Russian marmots (*S. guttatus* and *S. musivus*), *T. caviae* in the guinea-pig, exceedingly rare.

TRYPANOSOMATA OF BATRACHIANS

I. *T. rotatorium*.—Occurs in various species of frogs: *Rana esculenta*, *R. temporaria*, *Hyla arborea*, etc. It is characterised by extreme variation both in dimensions and in appearance. Thus França and Athias describe, in *R. esculenta* in Portugal, the following forms, which they consider to be distinct species, but the validity of these and many other trypanosomes with new names, is a matter of doubt.

Ia. *T. costatum*, Var. I:—Striated. About 45 μ by 25 μ . Blepharoplast about 2 μ from posterior end.

Nucleus close to it. Undulating membrane much folded. Flagellum, 10μ .

Ib. T. costatum, Var. II :—Striations, but not always well defined, 37 by 14μ . Blepharoplast, 10μ from posterior end. Nucleus close to it. Undulating membrane much folded. Flagellum, 24μ .

Ic. T. rotatorium (*s. strict*).—Non-striated, but very full of staining granules. 38μ by 18μ . Blepharoplast about 3μ from posterior end. Nucleus very long, 17μ by 2μ , 13μ from the anterior extremity. Undulating membrane much folded. Flagellum, 22- 30μ . During division it becomes round, losing its membrane and flagellum.

Id. T. inopinatum.—*Vide* below.

Ie. T. undulans.—32 by 7.5μ . Blepharoplast 10μ from posterior end. Nucleus about the middle. Usually no free flagellum.

If. T. gracile (S and C).—syn. *T. elegans* (F and A). 36 by 3μ . Longer and thinner than previous one. Free flagellum very short, about 3μ .

2. *T. mega*.—In a Gambian frog. 72μ by 8μ , not including the flagellum 10- 15μ .

3. *T. karyozeukton*.—In a Gambian frog. 57 by 3.5μ . Flagellum 15μ . A chain of granules joins the two nuclei.

4. *T. rotatorium*, var, *nana*.—In *R. esculenta*, Algeria. Average length, 22μ .

5. *T. inopinatum*.—In *R. esculenta* in Algeria, 25- 30μ , including flagellum. Is pathogenic to frogs, producing oedema, hydro-pericardium, ascites, haemorrhages. Trypanosomes are abundant in the blood. It has been thought that the trypanosome is a flagellate stage of one of the haemogregarines of the frog. Leeches (*Helobdella algira*) transmit the disease for as late as a month after their infection.

6. *T. somalense* in *Bufo reticulatus*. Somaliland. 30μ , flagellum, 7μ .

7. *T. nelspruitense*.—In frogs in the Transvaal, characterized by its long flagellum, $20-35\mu$, giving a total length of $40-70\mu$.

8. *T. borreli* (Marchoux and Salimberi), in *Hyla* sp. Brazil. Resembles *T. rotatorium*. No free flagellum.

9. *T. diemyctuli* (S and C).—In *Diemyctulus viridescens*, an American newt.

10. *T. belli*.—In a Hong-Kong frog. Two forms found, (a) 25μ , flagellum 16μ by 1.4μ broad, (b) length 22.6μ , flagellum 3μ by 1.9μ broad.

CULTIVATION OF FROG TRYPANOSOMES

Cultures grow in about five days. Sub-cultures may take several weeks to develop. Cultural forms are fusiform in shape, 25 by 2μ , with the blepharoplast anterior. Round forms 2μ in diameter also occur.

TRYPANOSOMES IN TORTOISES

1. *T. damoniae*.—In a fresh water tortoise (*Damonia reevesii*). It is intermediate in shape between *T. lewisi* and *T. rotatorium*. In the same tortoise also commonly occurs *Haemogregarina stepanowiana* and *H. rara*.

TRYPANOSOMES IN LIZARDS

1. *T. boueti* (Martin) 1907, in *Mabuia raddonii*, in French Guinea. Resembles *T. rotatorium*.

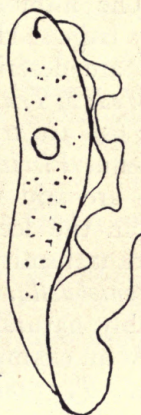
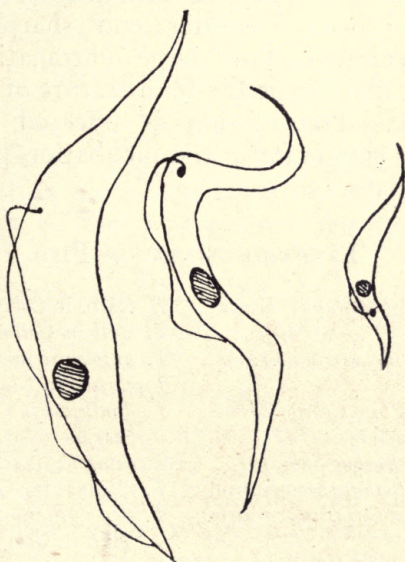
*T. costatum. Var. I**T. rotatorium**T. undulans**T. gracile (elegans)**T. inopinatum*

Fig. 110

TRYPANOSOMES OF FISH

The number of trypanosomes described in fish is very large. Here, again, indifferent male and female forms are described.

Mode of Transmission.—The trypanosomes of fresh-water fish are transmitted by leeches, *e.g.*, by those of the genus *Piscicola* and *Hemiclepsis*, and the trypanosomes are said to undergo a complex developmental cycle in the leech. The trypanosomes of saltwater fish are transmitted by leeches of the genus *Hemibdella* and *Pontobdella*. Leeches commonly contain flagellates, probably natural to them (cp. p. 361).

As an example of fish trypanosomes we may give:

1. *T. granulosum*.—In the eel (*Anguilla vulgaris*). They vary in size from 44-80 μ in length, and 2.5-3 μ in width. - Very active in their movements. Undulating membrane broad. Posterior end sharply pointed. Stained specimens show large chromatic granules. They live in blood kept at a temperature of 10 to 19° C. for a week. Transmission is effected by leeches (*Hemiclepsis marginata*); the incubation period being about five days.

TRYPANOSOMATA OF FISH

<i>T. abramis</i> in <i>Abramis brama</i> , bream	<i>T. cobitis</i> in <i>Cobitis fossilis</i> , loach
<i>T. acerinae</i> in <i>Acerina cernua</i> , pope	<i>T. cotti</i> in <i>Cottus bubalis</i>
<i>T. barbatulae</i> in <i>Cobitis barbatula</i> , loach	<i>T. angolensis</i> in <i>Claris angolensis</i>
<i>T. barbi</i> in <i>Barbus fluviatilis</i>	<i>T. clariae</i> in <i>Claris spp.</i> , China
<i>T. bothi</i> in <i>Bothus rhombus</i> , brill	<i>T. danilewskyi</i> in <i>Cyprinus carpio</i>
<i>T. carassii</i> in <i>Carassius carassius</i> , carp	<i>T. delagei</i> in <i>Blennius pholis</i>
<i>T. callionymi</i> in <i>Callionymus dracunculus</i>	<i>T. elegans</i> in <i>Gobio fluviatilis</i>
	<i>T. flesi</i> in <i>Flesus vulgaris</i> , flounder
	<i>T. gobii</i> in <i>Gobius niger</i>
	<i>T. langeroni</i> in <i>Cottus gobio</i> , miller's thumb

<i>T. laternae</i> in <i>Platophrys laternae</i>	<i>T. rhamdiae</i> in <i>Rhamdia quelen</i> , Brazil
<i>T. leucisci</i> in roach	<i>T. saccobranchi</i> in <i>Saccobranchus</i> <i>fossilis</i> , Ceylon
<i>T. limandae</i> in <i>Limanda plates-</i> <i>soides</i>	<i>T. scardinii</i> in <i>Scardinius</i> <i>erythrophthalmus</i>
<i>T. macrodonis</i> in <i>Macrodon mala-</i> <i>baricus</i> , Brazil	<i>T. scyllii</i> in <i>Scyllium canicula</i> and <i>S. stellare</i> , dog-fish
<i>T. phoxini</i> in <i>Phoxinus laevis</i> , minnow	<i>T. soleae</i> (Fig. 112) in <i>Solea</i> <i>vulgaris</i> , sole
<i>T. percae</i> in <i>Perca fluviatilis</i>	<i>T. squalii</i> in <i>Squalius cephalus</i> , chub
<i>T. platessae</i> in <i>Platessa vulgaris</i> , plaice	<i>T. tincae</i> in <i>Tinca tinca</i> , tench ray
<i>T. remaki</i> in <i>Esox lucius</i> , pike	
<i>T. rajae</i> in <i>Raja punctata</i> , etc.,	

In Indian fish :—*Ophiocephalus striatus*, *Macrones seenghala*,
M. tengara, *Trichogaster fasciatus*.

In Singhalese fish :—*Macrones cavasius* and *Gobius ginris*.

In Nile fish :—The noke (*Mugil*), the dabib (*Polypterus*), the
bagara (*Bageus bagard*), the gargur (*lynodontis schal*).

TRYPANOSOMATA OF BIRDS

1. *T. avium* (Laveran).—Occurs in the owl
(*Syrnium aluco*). 33-45 μ long (flagellum included).
The undulating membrane is well-developed, and has
several folds. The posterior extremity is pointed
(Fig. 112).

2. *T. johnstoni* (Dutton and Todd).—In the
blood of *Estrela estrela* in Gambia. It resembles a
spirochaete in appearance. There is no free flagellum.
36-38 μ long, by 1.4-1.6 μ broad.

3. *T. paddae* (Laveran and Mesnil).—In the
blood of *Padda oryzivora* (Java sparrow). 30-40 μ
long, by 5-7 μ broad. Posterior end very pointed.
Undulating membrane narrow and folded, but difficult
to stain. Division takes place longitudinally. Patho-
genic (?).

4. *T. confusum* (Lühe) = *T. avium* (Novy and McNeal).—In various North American birds, *e.g.* sparrow. *Large form*.—Total length, 50-85 μ ; *Small form*.—25-30 μ .

5. *T. laverani*, in *Astragalinus tristis*. North America.

6. *T. gambiae* (S and C), in Gambian birds. 8 μ broad.

7. *T. columbae* (S and C), in Indian pigeons, by Hanna. 45-60 by 6-8 μ .

8. *T. corvi* (S and C), in *Corvus splendens*, 40-56 by 3-5 μ .

9. *T. milvi* (S and C), in *Milvus govinda*, Indian kite. 34 by 3-4 μ .

10. *T. polyplectri*, Vassal, in *Polyplectron germani*, peacock pheasant, in Annam.

11. *T. bramae* (S and C), in *Athene brama*, an Indian owl.

12. *T. mesnili* (Novy and McNeal), in *Buteo teneos*, a buzzard. 50 by 8 μ .

13. *T. mathisi* (Ed. and Et. Sergeant), in *Hirundo rustica*, swallow. 22 by 4 μ . In culture Herpetomonas forms 20 by 2-3 μ , flagellum 17-25 μ . Has very large granules. Round forms also occur in cultures.

14. *T. bycanistis* (S and C), in *Bycanistes bucinator*, trumpeter hornbill.

15. *T. nicticoracis* (S and C), in *Nicticorax gardenia*. Brazil.

16. *T. asturinulae* (S and C), in a grey Congolese hawk, *Asturina monogrammica*.

Trypanosomes, unnamed and undescribed, occur in many other birds, *e.g.*, vultures, shrikes, thrush, blackbird, yellow hammer, rollers, blackcap, chaffinch, goldfinch, kingfisher, egrets, African doves and owls, etc., etc.

Detection of Trypanosomes.—The most certain method is by culture; this may be positive when repeated blood examination has been negative. Danilewsky states that, while rare in the blood (as is generally the case), trypanosomes may be abundant in the bone marrow.

TRYPANOSOMES OF INVERTEBRATES

1. *T. grayi*.—p. 342.
2. *T. tullochi*.—p. 343.
3. *T. piscicolae*.—Common in leeches, together with trypanoplasmata.
4. *T. christophersi*.—Found in one specimen of *Eu. sanguineus* (the Indian dog-tick). It is larger than *Herpetomonas* and *Crithidia* forms. 25 by 2.4μ . Flagellum $8-12\mu$. U.M. well developed. Blepharoplast close to the nucleus.

FLAGELLATES IN THE GUT OF INSECTS, ETC.

Besides the true trypanosomes natural to the tsetse fly already described, allied flagellates are found in the gut of various insects. These are generally assigned to two different genera, though the definition of these genera is at present somewhat vague.

Crithidia.—Includes two forms: (a) short, oval or pyriform flagellates, rounded posteriorly, somewhat truncated anteriorly, with a short straight flagellum ('pears'). The nucleus and blepharoplast are posterior.

(b) Longer forms, rounded at each end or tapering slightly at one end, (not at both as in *Herpetomonas*), with a long flagellum ('cigars'). The nucleus is median and the blepharoplast half-way between it and the anterior end. The flagellum may have a fine extension of the periplast over it. There is no

posterior diplosome. Examples of these forms are :—

1. *C. fasciculata* (Leger), in the gut of Anophelines, also in the larvae and pupae. (Crithidia forms are very common in the rectum of mosquitoes in the tropics.) Two forms occur: (a) small form, 3.5 by 2μ ; (b) large form, 7 by 3μ .

2. *C. minuta*.—In *Tabanus tergustinus*, shows, *a* and *b* forms.

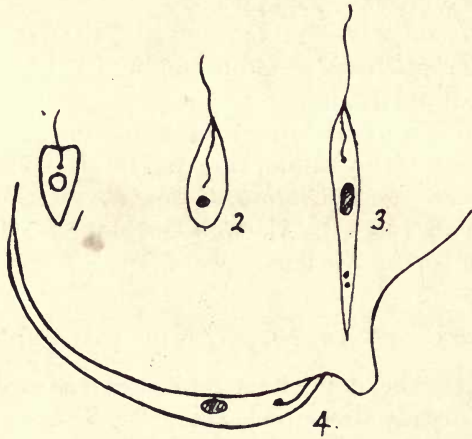


Fig. III. (1) *C. fasciculata*; (2) *C. minuta*; (3) *H. culicis*;
(4) *H. subulata*

3. *C. subulata*.—In the gut of Tabanidae.

4. *C. campanulata*.—In the larva of *Chironomus plumosus*.

Herpetomonas.—Body spindle-shaped, *i.e.*, tapering at both ends, with an ovoid nucleus centrally placed and a long sausage-shaped blepharoplast placed anteriorly. From this proceed two rod-like rhizoblasts, and at the end of these occur two granular points (diplosome). From these arise two flagella, which are united by a membrane. This double flagellum is not

visible in most *Herpetomonas* forms, so that practically a spindle-shaped flagellate, with anterior blepharoplast, is a *Herpetomonas*. The undulating membrane is, if present, only slightly developed and difficult to see. There is, therefore, little, if any difference, between a *Herpetomonas* and the forms taken on by trypanosomes in culture. Further, the *Herpetomonas* assumes a resting gregarine form, attached to epithelial cells of the gut by their flagella, *e.g.*, in the pyloric dilatation of the larva, developing into free forms in the mid-gut of the mosquito.

1. *H. muscae-domesticae* (Burnett) in the gut of house flies, *e.g.*, *Musca domestica*, *Homalomyia scalaris*, etc. Also a similar one in the gut of *Stomoxys calcitrans*.

2. *H. sarcophagae* (Prowazek), in *Sarcophaga haemorrhoidalis*, the meat-fly.

3. *H. algeriense*, in the gut of *C. pipiens* and *S. calopus*. It is 12 by 2.5μ , flagellum 4.5μ , blepharoplast posterior. Non-motile forms also occur, 5.5μ in diameter, with flagellum 17μ . Resting (gregarine) forms without flagellum also occur.

4. *H. culicis* (Novy).—In *C. pipiens*, etc. Actively motile: (1) long, 25-35 μ by 1-1.5 μ ; flagellum, 5-10 μ ; (2) medium, 15-25 by 1.5-2.5 μ ; flagellum, 3-8 μ (male type); (4) Wide form, 20 by 2.5-3 μ ; flagellum, 5-8 μ (female type). They all have a double body (diplosome) situate posteriorly (Fig. 111).

These forms also become spherical, about 5μ in diameter. *H. culicis* can be grown on Novy and McNeal's medium. The forms in culture resemble those in the mosquito.

5. *H. pulicis*.—In the gut of fleas; *P. cleopatrae* (Balfour) and *Gtenocephalus felis* (Patton).

6. *H. subulata*.—In *Tabanus glaucopsis* and *Hae-*

matopota italica. It is 30 by 1-2 μ , flagellum 20-25 μ . (Fig 111).

Dissect out the gut of a mosquito, *e.g.*, *C. fatigans*. Note with the naked eye (in an infected mosquito), the distension of the gut by the flagellates. Rupture the gut and make smears. Stain with Romanowsky.

Significance of Gut Flagellates.—In the case of *T. gambiense*, we know that all traces of trypanosomes disappear from the gut of the fly in ninety-six hours (but it is quite possible that they still exist in some unknown form).

Similarly, in the case of *T. brucei*, the trypanosomes disappear in fifty-five hours. On the other hand, in the case of fish trypanosomes, multiplication occurs in the leech, and they are infective for weeks.

It is necessary, therefore, to bear in mind the existence of these flagellates in the gut, sometimes in enormous numbers, especially as the cultural form of a trypanosome is of a herpetomonas type, *i.e.*, spindle shape, with anterior blepharoplast.

Pure cultures of gut flagellates are best got on blood-agar plates by a series of diluting smears.

GENUS TRYPANOPLASMA

Anteriorly has a long rod-like blepharoplast. Close beside this are two minute basal bodies, from which the flagella arise; the anterior free flagellum is attached anteriorly to one basal body, while the posterior, arising from the other, is attached along the whole length to the edge of the undulating membrane, and then terminates posteriorly in a free flagellum (Fig. 112).

Half a dozen of these flagellates are already known in the blood of fishes. They vary from 12-40 μ in length.

As in the case of trypanosomes, indifferent male (with large blepharoplast) and female (deeply staining) forms are described. Further, a developmental cycle occurs in certain leeches, *e.g.*, *Piscicola sp.*, with fertilisation, formation of ookinets, and development of three forms of trypanoplasmata out of these. As an example may be taken :—

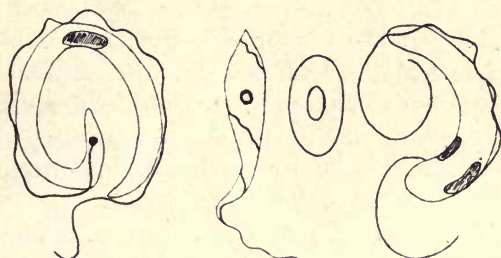


Fig. 112. *T. soleae*; *T. avium*; *Tp. borreli*

1. *Tp. borreli*.—Twenty μ long, three to four μ broad. Each flagellum, fifteen μ long. It is curved in shape. The undulating membrane is on the convexity. The anterior end is more pointed than the posterior. Found in the blood of the rudd (*Leuciscus erythrophthalmus*). Also a similar, if not identical, species in minnows (*Phoxinus laevis*). They may cause anaemia, wasting, and death of the fish (Fig. 112).

<i>Tp. abramidis</i> in <i>Abramis brama</i> ,	<i>Tp. truttae</i> in <i>Salmo fario</i> , trout
bream	<i>Tp. varium</i> in <i>Cobitis barbatula</i> ,
<i>Tp. barbi</i> in <i>Barbus fluviatilis</i>	loach
<i>Tp. guernei</i> in <i>Cottus gobio</i> ,	<i>Tp. cyprini</i> in <i>Cyprinus carpio</i> ,
bull-head	carp

LITERATURE

Laveran & Mesnil. *Trypanosomes and trypanosomiasis*. English Edition, 1907. Translated by D. Nabarro.

Chapter XXX

THE LEISHMAN-DONOVAN BODIES

Leishmania donovani

1. The parasites known by this name were discovered in the spleen in the disease so common in India, known as chronic 'malarial' cachexia, Damsdam fever, Kala-azar, tropical spleno-megaly, etc. They have since been found by splenic puncture in a few cases of chronic fever from Africa.

2. The same (?) parasites also have been found in the granulation tissue of Tropical ulcer, Delhi boil, Aleppo button, Scinde sore, Oriental sore, etc.

CLINICAL CHARACTERS OF THE FEVER

1. *Great enlargement of the spleen*, which frequently reaches the umbilicus and even the pubis. This is the most distinctive character of the disease.

2. *Emaciation*.—Usually present in advanced cases, and in fatal cases it is extreme.

3. *Irregular pyrexia*.—Uninfluenced by quinine, the accompanying chart indicates its character in a well-marked case.

4. *Abdominal symptoms*.—Dysenteric ulceration with blood and mucus in the stools in advanced cases. Death from peritonitis following perforation is not uncommon.

5. *Ulcerations*.—Cancrum oris, Noma vulvae, or other phagedaenic processes are common. Small

ulcers occur about the knees and elbows, or larger ulcers on the leg. The occurrence of these ulcers should arouse suspicion of a systemic infection with the parasite, for in Madras all cases affected with Noma or Cancrum oris yielded parasites on splenic puncture.

6. *Skin lesions*.—Especially in advanced cases, papular eruptions occur about the thighs and scrotum.

7. *Haemorrhages, epistaxis, petechiae, purpura*, etc.

8. *Oedema of the feet*.—Occasionally but not constantly present.

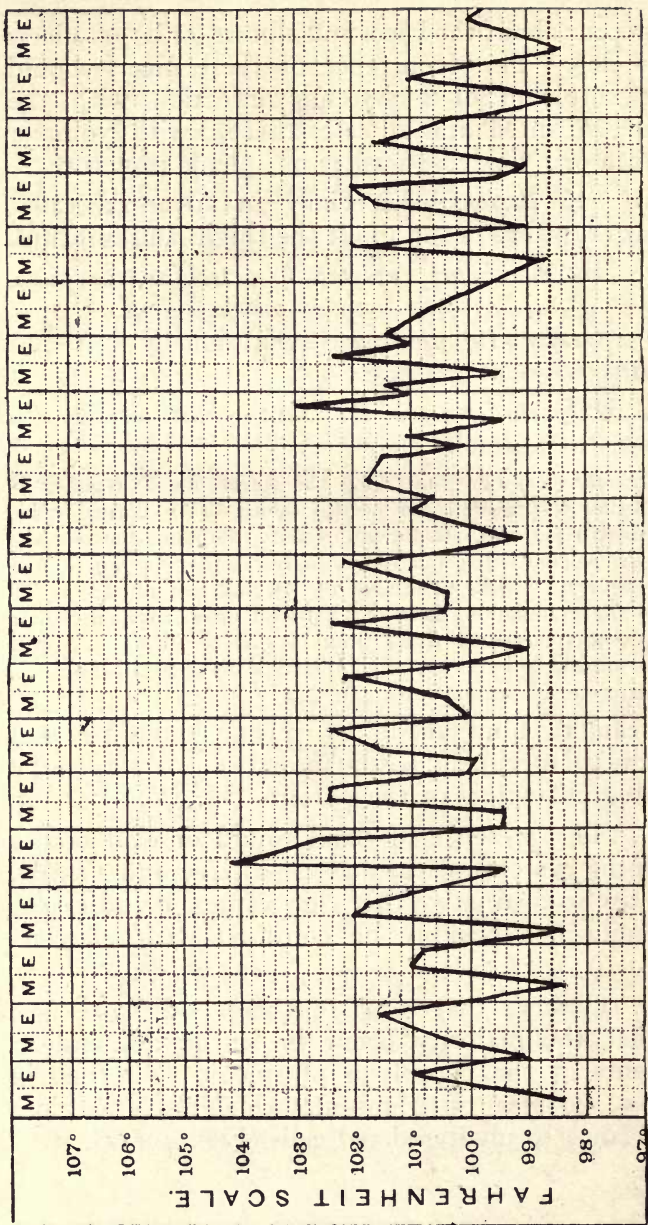
9. *Pigmentation of the skin*.—Not usually in excess of the normal.

Technique.—(1) For puncturing the spleen use a hypodermic needle. Boil it previously in normal saline, or in normal saline containing 0.1 per cent. ammonium oxalate. Puncture between the ribs if the splenic enlargement is not great, otherwise where it is most prominent. Make a number of dry and wet films. (2) To examine the granulation tissue of 'Tropical ulcer' snip off with a curved scissors pieces of tissue from papules or ulcers. Crush a fragment on a slide by means of another slide and make thin smears. Fix other pieces for section cutting (p. 44).

Examine films made by splenic puncture and in stained specimens (Romanowsky); observe the following characters of the parasite (Pl. IV):—

1. The presence of small round or oval bodies containing *two* chromatin masses—a large and a small. These are so distinctive that they cannot be mistaken, and could not possibly be confused with platelets (Figs. 1-6).

2. Observe that some of these bodies are free but that the majority occur in leucocytes, and in fragments of the cytoplasm of splenic cells (matrix of Ross, zooglea of Manson), which have a close resemblance to unaltered red cells (Figs. 12-14).



Advanced case of tropical splenomegaly. Spleen reaching to umbilicus.

3. Further observe that polynuclear leucocytes contain only one or two of these bodies (Fig. 16); large mononuclear leucocytes one to six (Fig. 17); cells of an endothelial type one to twelve (Fig. 18); large cells with a hyaline or finely granular or vacuolated cytoplasm (macrophages) up to several hundreds (Fig. 19).

4. The parasites are approximately circular or oval, $2.5-3.5 \mu$ in size, clearly outlined, and appear to possess a distinct cuticle, as they retain their shape and are rarely seen distorted in films.

5. The two chromatin masses are characteristic, the large one staining lightly and the small one intensely with Romanowsky. The masses are usually situate opposite each other in the short axis of the parasite. The larger chromatin mass always forms part of the periphery of the parasite.

6. Most of the parasites contain one or two vacuoles which may displace the cytoplasm of the parasites to the periphery.

7. Developmental forms. Division commences at the thick end of the parasite, and the large chromatin masses may be widely separated before the small chromatin mass has begun to divide. As many as three to six bodies are formed in this way, the large nuclei being arranged peripherally, and the smaller centrally (Figs. 7-11).

OCCURRENCE IN PERIPHERAL BLOOD

Parasites can be found in the blood in about eighty per cent., if not in all cases. Properly spread films (p. 19) must be used, especially as leucopenia is so common, and the leucocytes carefully examined; for the parasites do not occur free. It may be necessary to examine more than one film (Patton).

LEUCOCYTIC CHANGES

Leucopenia is the most marked change. So much is this so, that it is necessary to take several large films in order to make accurate leucocytic counts. Two thousand leucocytes per mm.³ is a common value and still smaller numbers are not uncommon. The *relative* leucocytic values, however, do not vary much from the normal, and are quite unlike those of malaria.

		Case I	Case II	Case III	Case IV
Large mononuclear	- -	11·4	8·6	10·6	16·0
Transitional	- -	1·8	0·6	—	—
Small mononuclear	- -	24·6	20·4	29·2	7·0
Myelocytes	- -	1·2	0·2	0·6	—
Polynuclear.	- -	60·0	69·8	56·0	73·0
Eosinophil	- -	1·0	0·4	3·6	4·0

POST-MORTEM CHANGES

Spleen.—The appearance of the spleen and the liver are almost pathognomonic. The spleen retains its shape when removed from the body as if hardened in situ. It is firm but friable, not tough like a fibroid spleen.

Liver.—Firm but friable, retaining its shape like the spleen on removal. On cutting into it an arborescent appearance is noticed, due to the deposit of a white tissue (macrophages containing parasites) in the centre of the lobules.

Large intestine.—Extensive multiple ulceration is almost constantly present. Fungating granulation tissue occurs in association with the ulcers. Purulent peritonitis, broncho-pneumonia, septic infarcts, are commonly met with. The other organs show no particular change to the naked eye.

MICROSCOPICAL CHANGES

1. Make *thin* smears of spleen pulp, liver, bone marrow, lung, kidney, testis, lymphatic gland, suprarenal. Stain with Romanowsky. Parasites occur in immense numbers in the spleen, liver, and bone marrow. To a less extent in the lungs and testis. They are present also in the suprarenals and lymphatic glands.

2. Make thin smears from granulation tissue of ulcers of the skin and intestine. Parasites are present in both situations; in the skin they are scanty, in the intestine they may be very numerous.

3. Place small pieces of these tissues on cover glasses. Fix in sublimate-alcohol (p. 404). Embed in paraffin. Cut sections. Stain with haematein. The study of sections is essential for a clear understanding of the relation of the parasite to the tissues. Observe the following conditions :—

Liver.—In the lumen of the capillaries of the lobule, often applied closely to the capillary wall, occur numerous large cells crowded with parasites. These cells are sometimes retracted and globular, but more usually they are characteristically extended, and suggest the idea that they are actually moving inside the capillaries. These cells are of doubtful nature but resemble the macrophages seen in the organs in malaria. In some cases these cells contain melanin. The parasites in these cells have the characteristic structure. They appear to lie in vacuoles, but these are undoubtedly the body of the parasites (Fig. 24).

Spleen.—The parasites occur in similar cells. They are very conspicuous in sections. Large mononuclear cells containing parasites are more abundant than in the liver. Neither do the red cells contain parasites, nor do free forms occur.

In contrast to what is seen in blood films made by spleen or liver puncture where most of the parasites are either free or contained in a matrix, in sections no such relation exists; the parasites lie in cells. These cells are of various types.

(a) But slightly modified endothelial cells. These have an oval nucleus and extensive protoplasm showing vacuolization (Fig. 20). The protoplasm may show buds or protrusions. These cells contain six to twelve parasites. Identical cells are seen in the capillaries of the testis and of granulation tissue.

(b) Large round cells with a large nucleus. The protoplasm has a ground glass appearance and is vacuolated. In the testis and in granulation tissue these cells are attached at one point to the capillary wall, the rest of the cell projecting freely. They also occur in the blood taken *post-mortem* from the large veins. They contain twenty or more parasites (Fig. 21).

(c) Very large cells with one or two vesicular nuclei. They occur in the liver and spleen in immense numbers. They occur either extended along the capillary wall or in a retracted form. In the spleen their processes extend among the smaller cells of the pulp. They contain numerous parasites.

(d) Large cells staining more intensely than the last and sometimes showing signs of necrosis. The nucleus is pushed to the side. The centre of the cell is occupied by a large vacuolated space, around which are arranged numerous parasites. The cells, in fact, contain so many parasites that they appear to be on the point of rupture, and such cells are rarely seen whole in films unless fixed extremely carefully with osmic acid vapour. They contain as many as two hundred and fifty bodies (Figs. 22, 23).

Bone marrow.—In films the parasites occur in macrophages in immense quantity. To some extent also in large mononuclear cells, and a few in polynuclear cells, and in myelocytes.

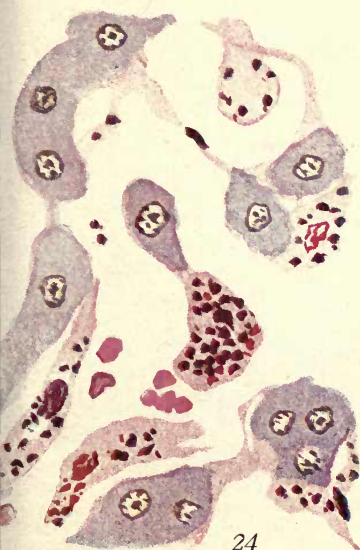
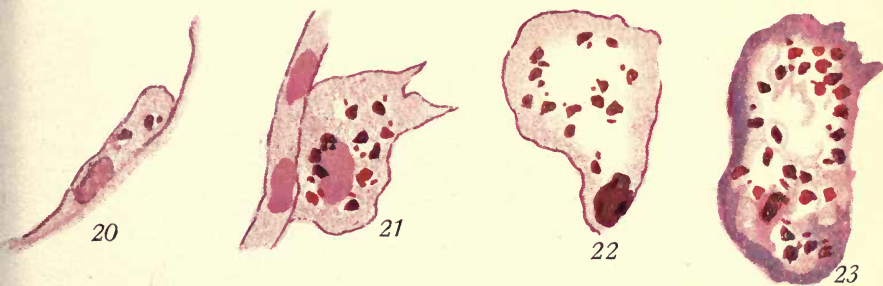
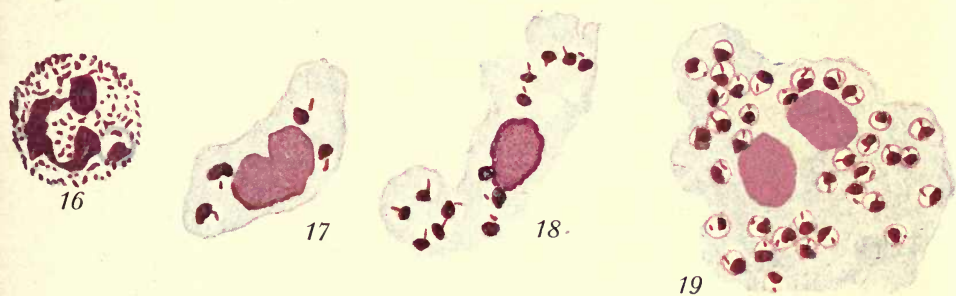
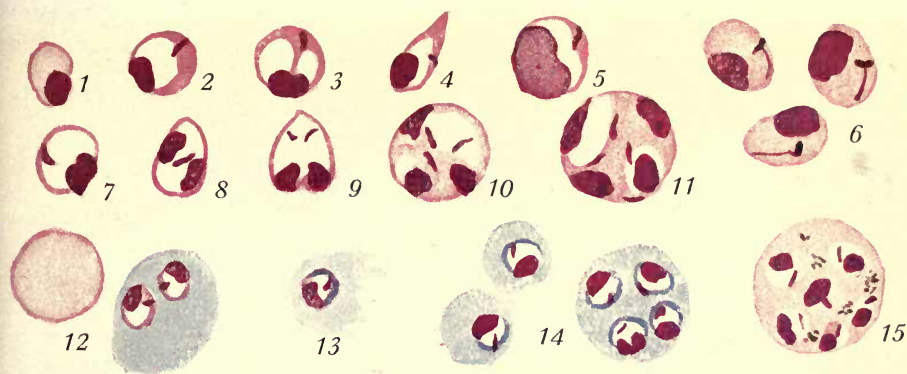
Large intestine.—Parasites occur in large numbers in the granulations, and in the mucous membrane in the early stages of infiltration. They occur in similar cells to those found in other situations.

Granulation tissue.—Sections of papules or ulcers of the skin show a few parasites in what are apparently endothelial cells of the fine capillaries. In larger capillaries cells may contain three or four parasites, while in small vessels large cells similar to those in the liver and spleen are found crowded with parasites. These cells are attached at one point to the capillary wall.

PLATE IV

THE LEISHMAN-DONOVAN BODIES

- Fig. 1.—A rare form of the parasite without vacuole or secondary chromatin body.
- Fig. 2.—Small form of parasite.
- Fig. 3.—Parasite with 'tail' joining the chromatin masses.
- Fig. 4.—Pear-shaped form.
- Fig. 5.—A large form with very large chromatin mass.
- Fig. 6.—Tail-like structures distinct from that of Fig. 3.
- Fig. 7-11.—Dividing forms.
- Fig. 12.—Parasites in apparently altered blood cells.
- Fig. 13.—Parasite in matrix, the remains of protoplasm of a leucocyte.
- Fig. 14.—Parasites in bodies after treatment with hypotonic ammonium oxalate solution.
- Fig. 15.—Parasites and pigment in apparently an altered red cell.
- Fig. 16.—Parasites in a polynuclear leucocyte.
- Fig. 17.—Parasites in a large mononuclear leucocyte.
- Fig. 18.—Endothelial cell containing parasites, from the femoral vein.
- Fig. 19.—A macrophage containing parasites.
- Fig. 20.—Endothelial cell from testis with parasites.
- Fig. 21.—Swollen endothelial cell from granulation tissue with parasites.
- Fig. 22.—Necrotic macrophage from spleen with parasites.
- Fig. 23.—A similar cell reduced to a mere pellicle with parasites.
- Fig. 24.—Section of liver shewing macrophages in the capillaries with parasites.
- Fig. 25.—Young granulation tissue from an ulcer shewing two parasites.



Lymphatic glands.—In those draining the area of a skin lesion parasites are found. They occur in large cells in the lymph sinuses and in cells of the reticulum.

Development.—Patton has traced the development of the parasite in the Indian bed bug *Cimex macropcephalus* up to the *Herpetomonas* stage, as seen in cultures.

Culture.—Add blood taken by a splenic puncture to a drop or two of ten per cent. sodium citrate in a small test tube. Slight acidification with citric acid

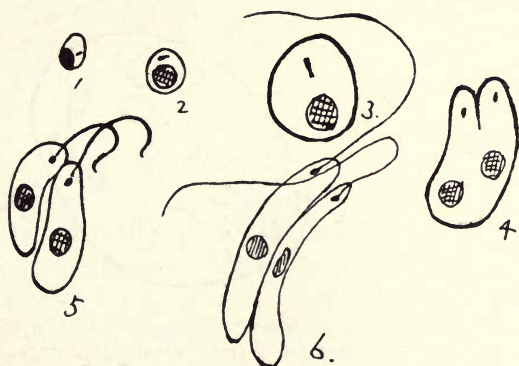


Fig. 113.—(1) Parasite as seen in splenic blood; (2) Third day, parasite enlarged; (3) Fourth day, full size; (4) Chromatin masses at the extremities; (5) Stumpy flagellate forms; (6) Elongate flagellate forms

assists development (Rogers). Incubate at 22° to 24°C . About the third day the parasites are as big as a red cell and the nuclei are no longer peripheral (Fig. 113). These forms may divide, forming groups, or flagella may develop directly from the enlarged parasite. About the fourth to sixth day elongate flagellates or forms still stumpy appear. They resemble *Herpetomonas* forms. Leishman describes also 'spirochaete' forms.

HISTOPLASMA CAPSULATA (Darling, 1906)

This parasite was found in enormous numbers in the tissues in a patient in Panama.

The Lungs shewed a condition closely resembling miliary tubercle but the nodules consist of broken down alveoli containing epithelial cells distended with parasites.

The Liver is enlarged. The liver cells and endothelium of the portal capillaries are invaded by myriads of parasites.

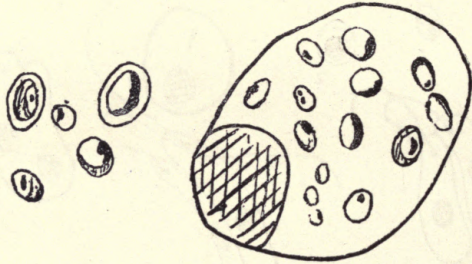


Fig. 114. *Histoplasma capsulata*: intracorpuseular forms from a smear of lung tissue, and free forms expressed from the cells

The Spleen is enlarged ($\times 3$) and firm. It contains many parasites.

Lymphatic Glands.—The mesenteric and splenic glands are enlarged, and parasites occur in mononuclear cells.

The Parasite is about half the diameter of a red cell. It is round or oval and is surrounded by a refractive rim. The parasite has a large and small staining mass. It is mainly an intracellular parasite. Whether it occurs in the blood is unknown.

Chapter XXXI

SPIROCHAETES

The Spirilla are distinguished from the spirochaetes: (1) by their inflexible body, (2) by the possession of a tuft of terminal flagella, (3) by their having a firm cuticular cell wall, as other bacteria, capable of resisting the action of ten per cent. potash, whereas spirochaetes are completely dissolved. What spirochaetes are, is a matter of great dispute. By some authors they are considered to be flagellates, by others bacteria.

Spirochaeta.—May provisionally be defined as follows:—Body not spiral (?) but shewing regular snake-like undulations in the same plane, consisting of sheath or periplast (? undulating membrane) and endoplasm. Division transverse (? also longitudinal), the two separating portions being often joined by a fine thread, the length of the two young individuals being equal to that of the original spirochaete. A terminal appendage (flagellum) can be shewn by special methods, but it may simply be a portion of the above thread or elongated sheath. Bacterium-like flagella exceedingly doubtful. They may be the frayed-out ectoplasmic sheath.

Encysted Forms.—Just before the crisis in the case of *Sp. duttoni*, Breinl described the formation of cysts in the spleen. The spirochaete becomes rolled up into tangles and is generally eaten up by phagocytes, but some few become encysted, the contents of the cyst breaking up into small granules. It is possibly from these granules that the new generation of spirochaetes develops.

The genus *Treponema* has a spiral body, not flattened, cylindrical in section, flagellum at each end. No undulating membrane, e.g., *T. pallidum* of syphilis and *T. pertenue* of yaws.

1. *Sp. recurrentis*.—The cause of relapsing fever in Europe. It remains to be seen whether the spirochaetes of relapsing fever elsewhere are identical with this or not.

Symptoms.—The attack lasts, as a rule, about six to seven days. The apyretic interval five to ten days. The following attack is shorter and the apyrexia longer. In two-thirds of the cases there may be a third attack, and even five or six relapses occur (cp. *S. duttoni*).

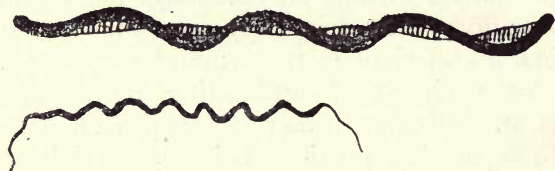


Fig. 115. (Upper figure) Plan of a spirochaete ;
(Lower figure) Plan of a treponeme (after Schaudinn)

Blood Examination.—Examine during the pyrexia a fresh film with a 1/6 lens. Note the slight disturbance among the corpuscles and, when attentively focussed, the gliding undulating movement of the parasite. If not found fresh, make a thick smear of blood on a slide as big as the area of a sixpenny bit. Dry thoroughly. Do not fix. Stain with gentian violet (a few drops of a saturated alcoholic solution to a teaspoonful of water) for about five minutes or less, or with Romanowsky. Parasites are generally numerous during the pyrexia, but disappear completely during the apyretic interval.

Fig. 4



Fig. 14

Fig. 3

Fig. 2



Fig. 9



Fig. 10

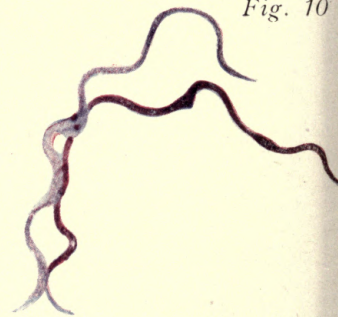


Fig. 11



Fig. 13



Fig. 15

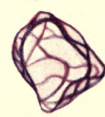


Fig. 16

Fig. 1

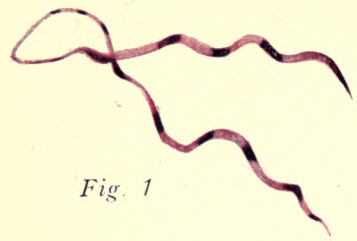


Fig. 6



Fig. 5



Fig. 8



Fig. 11

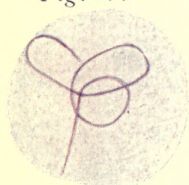


Fig. 12



PLATE V

Spirochaeta duttoni

Figs. 1, 2.—Spirochaetes shewing chromatic areas.

Fig. 3.—Irregular distribution of chromatin.

Figs. 4, 5.—Swellings in the spirochaete.

Fig. 6.—Unstained transverse band.

Fig. 7, 8.—Stages in transverse division.

Fig. 9.—Longitudinal division.

Fig. 10.—Conjugation (?).

Fig. 11.—Intracorpuseular spirochaete.

Fig. 12.—Coiled form in the peripheral blood.

Fig. 13.—Swollen form in the liver.

Figs. 14, 15.—Skein-like forms in the spleen.

Fig. 16.—Encysted form.

Morphology.—Length, on an average, 20μ , by 0.4μ broad; undulations six to eight in number, about 2μ between the summits of each. They stain uniformly, with the exception that frequently a minute clear spot can be seen about the middle of the length.

Mode of Transmission.—Is unknown, but in spite of negative experimental evidence, bugs or lice are the probable carriers.

Pathogenicity.—In monkeys the incubation period varies from one to four days, depending on the dose inoculated. The attack lasts two to six days, and there is usually one relapse. Rats and mice cannot, as a rule, be infected directly by human blood. A passage through a monkey is first necessary. In rats, the incubation period varies from a few hours to a few days. The attack lasts one to three days. Spirochaetes are scanty. Relapses occur, but last only a day. Rats always recover.

2. *Sp. duttoni.*—The cause of African tick fever.

Symptoms.—The attack lasts one to four days. The apyretic interval lasts about three days. The usual number of relapses is uncertain, they may be as many as six.

Blood Examination.—Spirochaetes, even at the height of the fever, are nearly always scanty.

Morphology.—The spirochaeta is, when fully grown, about 24μ by 0.45μ broad; the undulations, eight to ten in number, 2.2μ in width. (*Vide* Plate V.)

Pathogenicity.—It differs from *S. recurrentis* in that nearly all the ordinary laboratory animals, except cats, can be infected. Rats are most easily infected. They frequently shew immense numbers of parasites, and large tangles can be found. Several relapses may occur, unless the animal succumbs. An animal that has recovered from an attack of *S. recurrentis* is still

susceptible to inoculation with *S. duttoni*, and *vice versa*.

Pathology.—In animals the chief change is in the spleen, which is much enlarged, with haemorrhagic infarcts and areas of necrosis in chronic cases. There may be effusion into the serous cavities, and petechiae of the membranes. Necrotic areas occur in the liver, and the lymphatic glands are often haemorrhagic. The bone marrow is soft.

Mode of Transmission.—This is effected by *Ornithodoros moubata* (p. 329) in the adult and nymphal stage, the latter being probably by far the most important. The transmission is thus hereditary. The incubation period from tick bites, in the case of animals, is about five days. Koch found from seven to fifty per cent. of ticks collected from native huts, infected. Koch found spirochaetes in about twenty-five per cent. of eggs up to the twentieth day of development in the ovaries of ticks which had sucked spirochaete blood.

ADDENDUM

The spirochaete in East African tick fever is morphologically identical but different from *S. duttoni*, according to Fränkel.

3. *Sp. novyi*.—Uhlenhuth and Haendel have shewn, by means of agglutination with specific spirochaete sera, that the American relapsing fever is different from others.

It is about 17μ long by 0.3μ broad, the undulations, six to eight in number, 1.9μ in width.

4. *Sp. carteri* (Mackie) of Bombay relapsing fever, the mortality of which is 38 per cent. The commonest forms are 10 to 16μ , average length 26 to 32μ by 0.5μ . Monkeys are the most susceptible, but according to Mackie other animals can also be infected at least temporarily.

AGGLUTINATION OF SPIROCHAETES

Inject a rabbit, intravenously, on two or three occasions with $\frac{1}{2}$ -1 c.c. of spirochaete blood. Its serum will now agglutinate, render motionless and transform into granules the corresponding spirochaete, but has no action on other spirochaetes. Agglutination will occur up to dilutions of 1 in 100.

PFEIFFER'S PHENOMENON

Inject a mouse, intraperitoneally, with 0.1 c.c. of a specific spirochaete serum and $\frac{1}{4}$ c.c. of blood containing the corresponding spirochaete. The spirochaetes disappear from the peritoneal cavity in ten minutes, and the mouse does *not* become infected. If any other but the corresponding spirochaete be used, the spirochaetes are not destroyed and the mouse becomes infected in twenty-four hours.

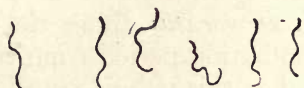


Fig. 116 *Sp. laverani*

5. *Sp. laverani* (Breinl, 1907).—In the blood of white and wild rats. In the fresh state it is an extremely small, active spirochaete, shooting across the field, revolving, turning, like a very active motile vibrio, and not like other spirochaetes. Stained, it has two to six spirals and is $2-6\mu$ in length (Fig. 116).

They are transmissible to mice and young rats only. The infection may persist for months, though the number of spirochaetes is then very small.

Incubation Period.—Five to six days, the infection reaches a maximum in about ten days. Spirochaetes also occasionally occur in the blood of *Mus decumanus*, and, it is stated, in the blood of bandicoots (*Nesocia bandicota*).

6. *Sp. muris*, var. *virginiana*, in a wild rat in Virginia, 3·5 by 0·25 μ . Easily transmissible by inoculation to wild rat, the incubation period being seven to fourteen days.

7. *Sp. theileri*.—Occurs in cattle in Africa (Transvaal and Cameroons). According to some the same spirochaete exists also in horses and sheep.

Symptoms.—As *Piroplasma sp.* and *T. theileri* are also commonly present, it is difficult to ascribe the affection and death solely to the spirochaetes.

Morphology.—The spirochaetes are actively motile, 20-30 μ long. Small forms, 8 μ , also occur.

Pathogenicity.—Besides cattle, sheep are susceptible.

Mode of Transmission.—The larva of the infected mother tick *M. decoloratus* (blue tick), transmits the disease. The incubation period from tick bite is about a fortnight; and as this tick passes all its life, three to four weeks, on cattle, it is possible for the larval tick to re-infect itself when adult, from the beast it has infected when itself in the larval stage.

The blood of immune animals is still infective (cp. *P. parvum*).

8. *Sp. anserina*.—The cause of a fatal disease in geese in the Caucasus and in Tunis.

Symptoms.—(1) Fever, (2) diarrhoea, (3) acute tenderness of the feet. Death occurs in about a week. The mortality is about eighty per cent.

Blood Examination.—The spirochaetes occur in

immense numbers, and tangles are common; they disappear again before death.

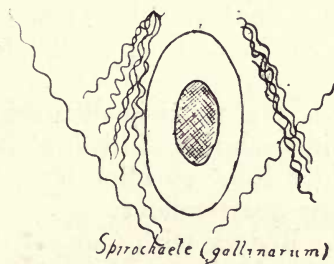
Morphology.—10-20 μ long. The spirochaetes are said to be surrounded with flagella.

Pathogenicity.—Young chickens and ducks to a less extent are susceptible.

Pathology.—The spleen is soft, and there are caseous foci in the liver.

9. *Sp. gallinarum*.—The cause of a fatal disease of poultry in Brazil, and probably in many other countries.

Symptoms.—(1) Fever, (2) acute diarrhoea, (3) anaemia, (4) somnolence, (5) convulsions. Death in four to five days, or the hens may die in a condition of cachexia in about a fortnight.



Spirochaete (gallinarum)

Fig. 117

Blood Examination.—During the pyrexia, parasites get more and more abundant, forming thick tangles. They disappear again, frequently quite suddenly, before death.

Morphology.—10-20 μ long, with many undulations (Fig. 117). According to some authors the spirochaete is surrounded with flagella; while Prowazek, on the contrary, describes an undulating membrane in macerated specimens.

Pathogenicity.—Geese, ducks, doves, sparrows and rabbits (slight infection) are susceptible.

Pathology.—The spleen is much enlarged and soft. The liver shows necrotic foci due, according to Levaditi, to an actual invasion of the tissue by spirochaetes. Stain sections of liver by Levaditi's method.

Mode of Transmission.—This is effected by *Argas miniatus*. This tick is a night feeder. A temperature of about 35° C. is necessary for successful transmission. The ticks remain infective for one month after feeding.

To Stain Spirochaetes in Sections.—Levaditi and Manoulian* give the following as their latest formula.

1. Fix small pieces of tissue, 1 millimetre thick, in formalin. Harden in ninety-five per cent. alcohol one hour. Then into distilled water until they sink.

2. Put into a solution of one per cent. tannin to which enough pyridin is added to clear up the turbidity arising on first mixing. Keep in this for one-quarter hour at 50° C.

3. Wash several times in distilled water.

4. Place in a solution of silver nitrate 1 per cent., to which is added some pyridine ten per cent. Keep at 50° C. for one-quarter hour.

5. Wash. Reduce with four per cent. pyrogallie acid, to which is added enough pyridine to make the mixture clear. Reduction takes place in a few minutes.

6. Wash, alcohol, xylol, paraffin. Cut sections.

7. Double stain with neutral red and methyl blue.

10. *Sp. vespertilionis*.—In a Tunisian bat, *V. kubli*. The infection is sometimes fatal, and relapses occur. The incubation period, after inoculation, is about forty-eight hours. The spirochaete is 12-18 μ . They disappear out of the blood by crisis.

The spirochaete, according to Gonder, divides

longitudinally and has an undulating membrane (periplast), which is continued at each end in the form of a flagellum. Intracorpuseular forms are also described. Transmission is probably by ticks (Ixodine).

OTHER SPIROCHAETES

1. *Sp. equi*.—In the horse in French Guinea. 12-15 μ , with three to four undulations.

2. *Sp. ovina*.—In sheep in Erythræa, possibly identical with *S. theileri*.

3. *Sp. gondii*.—In the gundi, *Ctenodactylus gondi*, N. Africa.

4. *Sp. jonesi*.—In an African fish (*Clarias angolensis*). Has a rigid appearance, unlike a spirochaete.

5. Spirochaetes have recently been cultivated by Topfer from the blood of owls (? species). Owls not uncommonly also contain trypanosomes.

SPIROCHAETES IN INSECTS

Sp. culicis (Jaffé).—In the gut of larvae of *Culex* sp. in temperate zones.

A. maculipennis.—In the gut of the larva, 8-17 μ , with one to four nodes.

C. pipiens.—(1) In the *malpighian tubes* in abundance (after feeding on blood), 25-30 μ , with three to eight nodes. (2) *In the gut*, one species with two to three nodes, and another species, 15-25 μ , with five to ten nodes.

Chironomus plumosus.—In the gut, 15-20 μ , with four to five nodes.

Glossina spp.—Also in the gut.

Chapter XXXII

YELLOW FEVER

The cause of the disease is unknown. The following facts have been established :—

(1) *Stegomyia calopus* that has fed on a patient during the first three days of the fever can transmit the disease twelve days later, but not before. This period is longer if the temperature of the air is low (*e.g.* 80° F.).

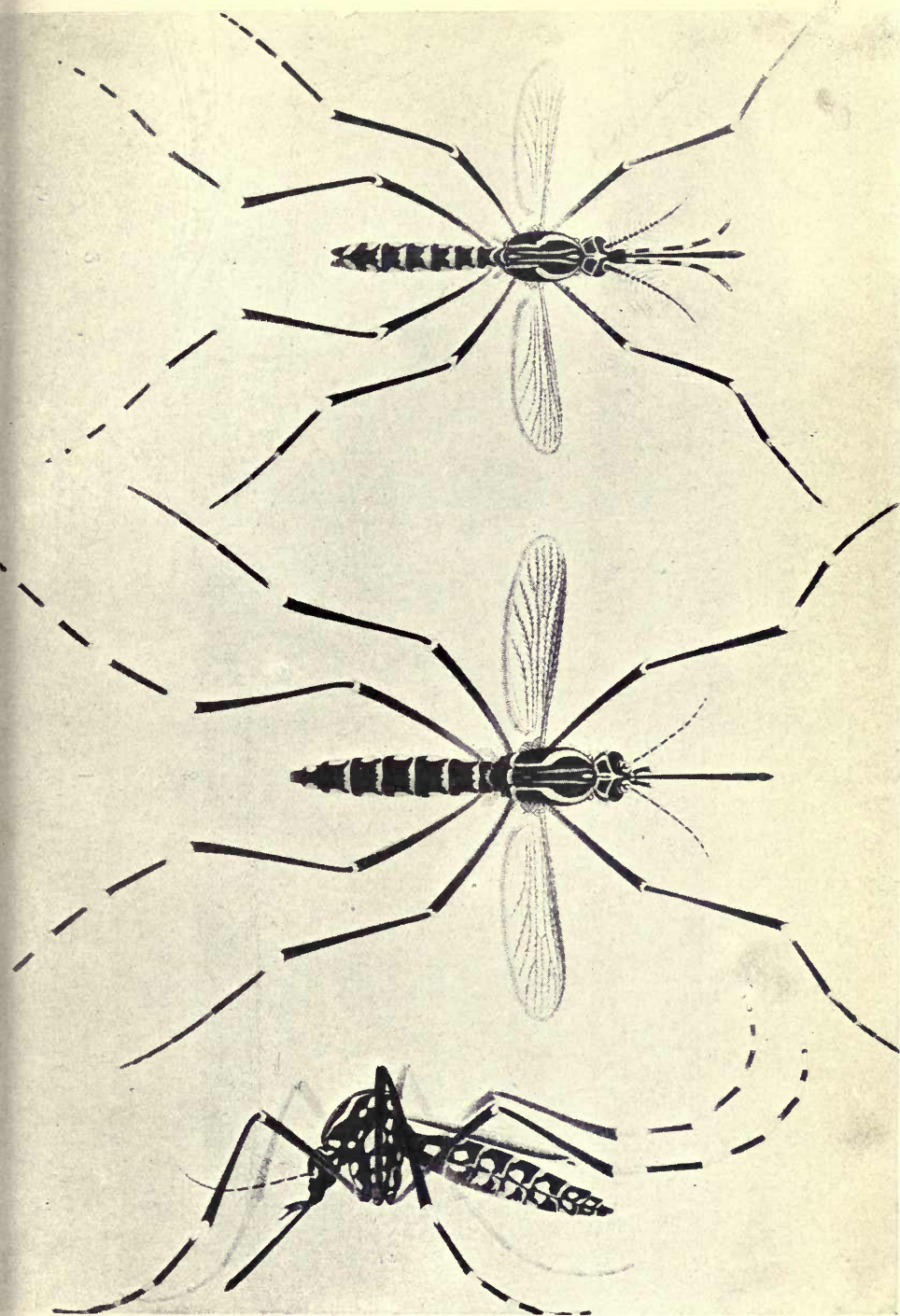
(2) The incubation period in the patient is two to six days.

Stegomyia calopus.—A black and white mosquito. Proboscis unbanded. Thorax ornamented with a curved silvery line on each side and two median parallel lines, giving the characteristic lyre pattern. Hind tarsi basally banded with white, the last tarsus being all white (Plate VI).

Habits.—According to some observers this mosquito, after the first week of life, bites only at night (5 p.m. to 7 a.m.), but this is doubtful, though probably feeding generally takes place at night.

Breeding Places.—Breeds especially in ‘domestic’ collections of water in pots, pans, barrels, jars, tins, cisterns, boats, troughs, etc., etc., but not commonly in natural puddles.

Egg-laying.—Occurs chiefly at night, sixty to seventy being laid. They measure 550 by 160 μ (p. 67). The eggs will hatch after being kept ‘dry’ for some months. Hibernation is probably mainly by the eggs.



Stegomyia calopus, male and female (dorsal and lateral view) after Goeldi

(By kind permission of the Crown Agents for the Colonies)

At 86° F. they hatch in thirty-six hours, but at 68° F. they will not hatch at all.

Larva.—(p. 81). This stage lasts about a week.

Nymph.—This stage lasts thirty-six hours.

Imago.—*In the absence of water*, in the tropics, it lives only three to four days. It is most active at 86° F., and is very sluggish at 68° F., and at 58° F., rarely bites.

The adults may be kept alive for nearly six months.

Chapter XXXIII

BLOOD-SUCKING FLIES

The Diptera or flies are two-winged insects (the posterior pair of wings being transformed into *halteres*), and are so distinguished for example from the Hemiptera or bugs which generally have four wings. In the Diptera the metamorphosis is complete, eggs, larva, pupa, insect; in the Hemiptera it is not so. The following have blood-sucking habits:—

The *Nematocera* (νημα, thread; κέρας, antenna).

1. *Blepharoceridae*.

Wings iridescent, ample, bare, with creases, no 'discal' cell on wing (the discal cell lies between the second posterior cell and the second basal cell). Posterior tibiae with stout spines, anterior tibiae unarmed. The fourth vein is the one immediately preceding the large posterior fork, the incomplete vein not being counted. They resemble midges. The larvae have suckers, and are found attached to stones in the water.

(1) Genus *Curupira* (? blood-sucking). — No incomplete vein between the first and fourth. Eyes contiguous. *C. torrentium*, Brazil.

(2) Genus *Snowia* (? blood-sucking). — Eyes separated by a broad frons. Palpi four-jointed, well developed.

2. *Culicidae*.—Mosquitoes or gnats.3. *Chironomidae*. (Midges).

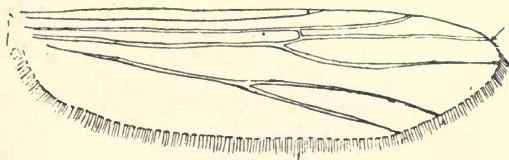
Head small, often retracted under thorax, which has no transverse suture. Simple eyes (ocelli) absent

or rudimentary. Antennae up to fifteen segments, densely pectinate in ♂, often simple in ♀ and smaller. Legs long and slender. Tibiae and tarsi nearly cylindrical. Costal vein ends at apex of wing.

(1) Genus *Chironomus*. — Not blood sucking. Larvae are 'blood worms,' 'Vers de Vase.'

(2) Genus *Ceratopogon*. — Very minute midges. Wings generally spotted. Head depressed in front, produced into a short rostrum. Antennae thirteen segments, the first eight bead-like, the rest elliptical. Sub-costal vein ends beyond half the length of the wing. Second long vein ends near the tip, third long vein at the tip. Femora armed beneath with spines. Larvae mostly non-aquatic. *C. varius*. A pest in Great Britain.

(3) Genus *Tersesthes*. New Mexico.



Wing of *Chironomus plumosus*.

Fig. 118. The arrow indicates the point at which the costal vein ends

4. *Psychodidae*. (Moth flies).

Very small. Antennae very hairy. Wings very hairy (*Vide* Fig. 17). Larvae of some genera amphibious. The larvae and pupae resemble those of *Ceratopogon*. The eggs are laid in a cluster on the water.

(1) Genus *Phlebotomus*. Europe and tropics.

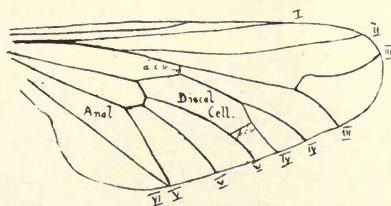
5. *Simulidae* (Sandflies, Buffalo-gnats).

Small hump-backed flies. Antennae destitute of hairs. Wings relatively large. Proboscis short, thick,

The *Brachycera* (βραχύς short, κέρας antenna) include the following :—

1. *Tabanidae* (Horse-flies or gad (= sting) flies).

Large flies. Antenna three-jointed, not terminating in a style or arista (the arista (when bristle-like) or style (when thick) being an appendage of the terminal portion (flagellum) of the antenna). Third segment of antenna annulated. Labium enclosing four stylets in ♂, six in ♀. The terminal joint of the palpi is inflated, and the palpi hang down in front of the proboscis. Eyes in ♂ holoptic (contiguous), occupying most of head area. In ♀ dichoptic (separate). The male fly does not bite.



Wing of *Hadrus* sp

Fig. 120. Wing of *Lepidoselaga* (*Hadrus*), a *Tabanid*. a.c.v. = anterior cross vein ; p.c.v. = posterior cross vein

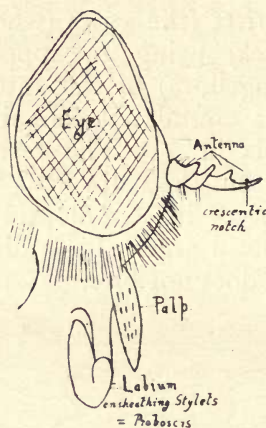
Egg.—Spindle-shaped. They are laid in spherical or flat groups on the stems of grass, etc.

Larva.—Are aquatic or live in damp earth. They are carnivorous. They are about an inch long.

Pupa.—Aquatic or terrestrial. Over an inch long.

The *Tabanidae* are divided into two divisions, comprising more than thirty genera and over thirteen hundred species. It is only possible to mention here the commonest genera.

1. Hind tibiae with spurs at the tip ; ocelli in most cases present. *Pangoninae*.
2. Hind tibiae without spurs at the tip ; no ocelli (simple eyes). *Tabaninae*.



Head of *Tabanus* sp. (after DeLong) *Head*

Fig. 121

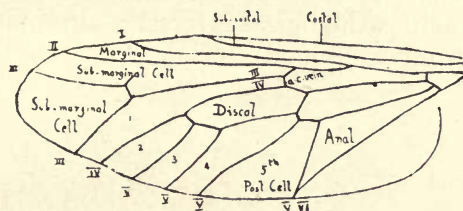
1. *Pangoninae*

(1) Genus *Pangonia*.—Face and front in ♀ *without* tubercles or callosities. Proboscis often long, thin, horizontal. In some, three to four times length of body, piercing, even when the fly is on the wing. Third segment of antenna, eight rings. Species about two hundred and fifty.

(2) Genus *Chrysops*.—Front *with* a tubercle or callosity. Three ocelli. Second segment of antenna almost as long as first. Eyes golden green. Flight silent. Wings widely separated ; spotted. Hind tibiae spurred. Species about a hundred and fifty.

Ch. caecutiens attacks the eyes especially.

(3) Genus *Silvius*.—Second antennal segment very much shorter than first. Wings without any spots. Third antennal segment five rings as in *Chrysops*. Species about twenty-six.



Wing of *Haemolopota pluvialis*.

Fig. 122

2. *Tabaninae*

The two most numerous genera are *Tabanus* and *Haematopota*.

(a) Front much longer than broad. Frontal tubercle when present not transverse.

(1) Genus *Tabanus*.—Proboscis short and thick ; vertical in the female, oblique in the male. Antennae scarcely longer than head. Third segment five rings. First ring is characteristically notched in shape of a crescent with a basal process (*Vide* Fig. 121). Eyes bare. Large flies with humming flight. Species about a thousand.

(b) Front as broad as it is long or broader. Frontal tubercle transverse, about four times as broad as long.

(2) Genus *Haematopota*.—Terminal segment of antennae not crescentic. Third segment has four rings. Wings adjacent like the side of a roof. They have transparent markings. No ocelli. Flight silent. About fifty species.

H. pluvialis. Common in woody lanes in England in the summer.

The third group (*Cyclorrhapha Schizophora*) include the *Muscinae*, *Sarcophagidae*, and *Oestridae*. The last two groups are not blood-suckers, but are included here for their pathological interest; also only some of the first group are blood-sucking.

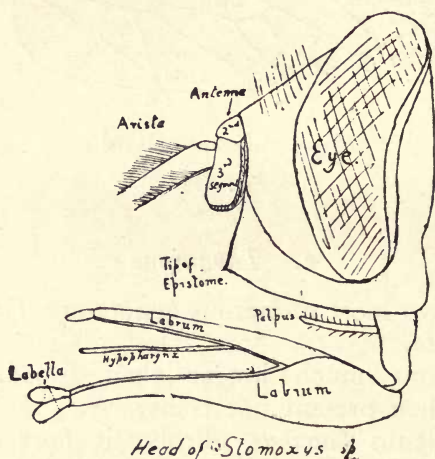


Fig. 123

1. *Muscinae* = *Muscidae* (restricted).

Antennae *dependent* in front of head. They have three segments. The third segment is flattened and pod-like in shape, with an arista plumose generally to the tip. Hind body devoid of stiff bristles.

(a) Genus *Musca*.—House flies (not blood-suckers).

(b) Genus *Calliphora*.—Blow flies or blue bottles (not blood-sucking).

(c) Genus *Lucilia*.—Green bottles (not blood-sucking).

L. macellaria.—The larva of this fly is the American 'screw worm,' infesting the nasal fossae and frontal sinuses of man.

(d) Genus *Auchmeromyia*.—*A. luteola*. The larva is the blood-sucking floor maggot of the Congo, etc.

BLOOD-SUCKING GENERA

(1) Genus *Haematobia*.—(a) Palpi shorter than proboscis, partly ensheathing it. (b) Labella fleshy, easily visible. (c) Arista plumose dorsally; three to four hairs ventrally. (b) Third and fourth long veins reach the apex of wing. Small mottled flies.

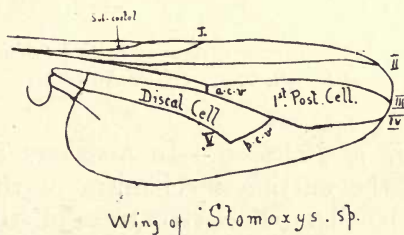


Fig. 124. Shewing the first posterior cell open at the margin of the wing

(2) Genus *Lyperosia*.—(a) Palpi long and flattened, ensheathing the proboscis. (b) Arista plumose dorsally. (c) Wing as in *Stomoxys*. Differs thus from *Glossina*. These flies are common on camels.

L. irritans.—Is the 'horn-fly' termed *H. serrata* in U.S.A.

(3) Genus *Beccarimya*.—(a) Palpi shorter than proboscis. (b) The first post-cell of the wing is closed before the margin.

(4) Genus *Stomoxys*.—(a) Palpi very small, bearing some hairs; not projecting beyond the epistome. (b) Proboscis is bent at its base like an elbow joint. (c) Arista plumose dorsally, distally forms a fine hair. (d) Third and fourth long veins reach the apex. The fourth is bent beyond the posterior cross vein. Wings diverge widely.

S. calcitrans.—The 'stable' fly, is common about farm yards.



Fig. 125. *Stomoxys*, shewing resting position of Wings, $\times 2$. (After Austen)

Structure of Proboscis.—In *Stomoxys calcitrans* as in *Glossina* the cutting mechanism of the proboscis lies in the labella. The structure of these is even more complex than in *Glossina*, but consists essentially of five very strong teeth on each side. These can be completely everted and by rotation of the proboscis tear through the skin. The proboscis proper consists of (a) labrum, (b) labium, and (c) hypopharynx. The relationship of these parts is shewn in the figure.

(5) Genus *Glossina* (*tsetse-flies*).^{*} — Abdomen generally, but not always, has pale but well-marked dark-brown bands interrupted in the middle.

^{*} The data of this section are compiled from *A Monograph of the Tsetse Flies*, by E. E. Austen, and from an article in the *British Medical Journal*, September 17, 1904, by E. E. Austen.

1. Dull-coloured, brownish flies, seven to twelve mm. long (excluding proboscis and wings).

2. Wings in resting position, closed flat, one over the other, scissors-like, projecting beyond the abdomen.

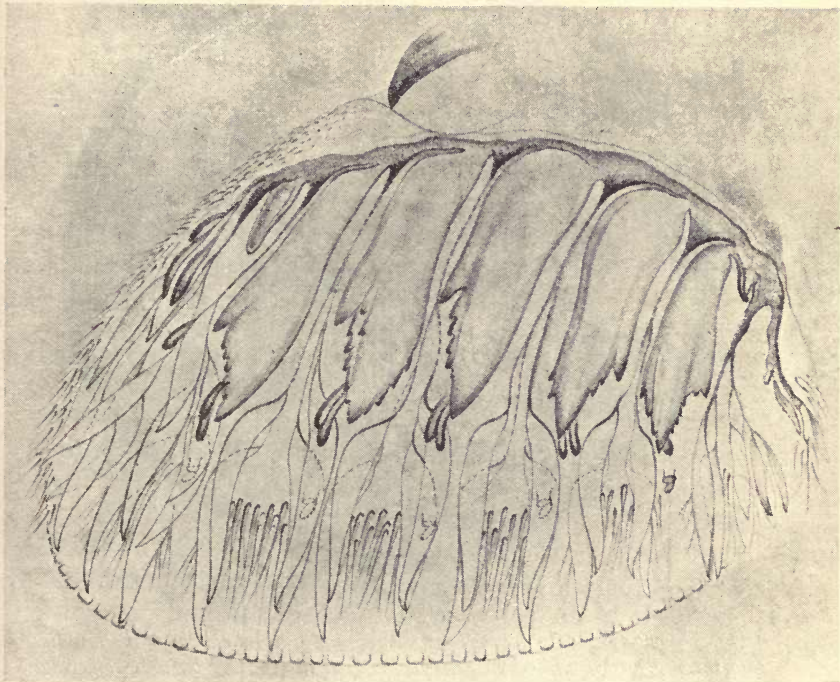


Fig. 125A.

(1). *Stomoxys calcitrans*. Inner surface of the labellum shewing five cutting teeth and various other structures. These teeth are completely everted during the act of biting.

(After Stephens and Newstead)

3. Proboscis ensheathed in palpi, projecting horizontally in front.

4. Base of proboscis suddenly expanded into a large onion-shaped bulb.
5. Arista feathered on upper side only.

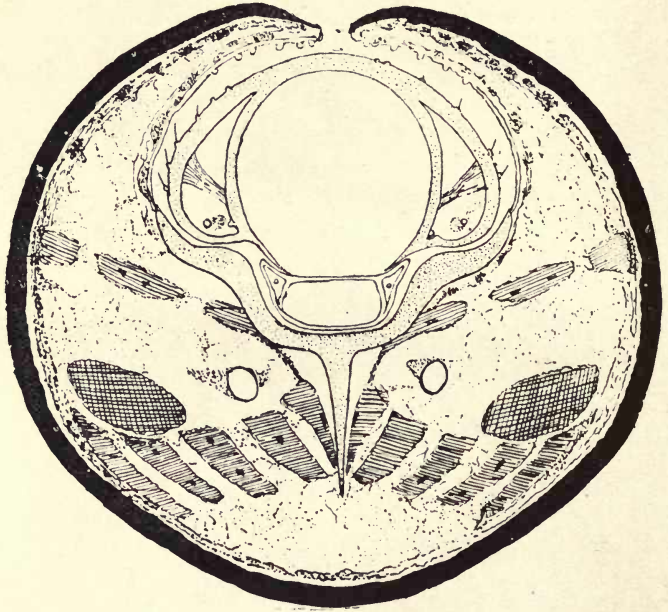


Fig. 125A.

(2). *Stomoxys calcitrans*. Transverse section through about the middle of the proboscis. The hypopharynx is kept in its place by the projecting ends of the labrum. The interlocking teeth of the labrum and labium are also shown

6. Male genitalia (hypopygium) highly characteristic, oval and tumid, with a vulviform median groove (anus) running from anterior margin to beyond the middle. Sex easily distinguished by this mark.
7. Wings absolutely characteristic, especially in the course of the fourth longitudinal vein (*vide* Fig.

126, iv). The anterior transverse vein is very oblique. The bend in the course of the fourth vein, before it meets the anterior transverse vein, is absolutely diagnostic.

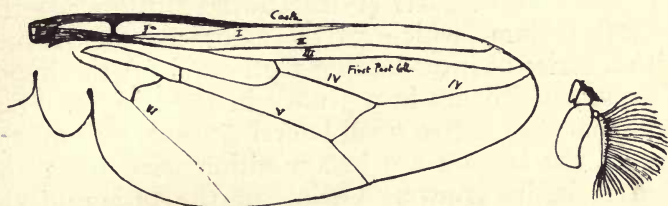


Fig. 126. *Wing Venation of Glossina, and antenna with Feathered Arista. (After Austen)*

LIFE-HISTORY

Tsetse flies are not found far from water. They frequent areas of thick wood or undergrowth, and are not found in open spaces or clearings. As a rule, tsetses and big game are found together, but there appear to be exceptions to this rule.* They have a great dislike for excrement of animals.

Tsetse flies do not lay eggs, but yellow-coloured larvae. These have been found among the roots of banana trees. After a few hours these change into pupae. The pupa is about six mm. long and three mm. broad, and consists of twelve segments. The twelfth segment is produced into two large lips, enclosing a pit, the site of the respiratory stigmata in the larva. These lips differ in shape and size in the different pupae. At the anterior end is a longitudinal groove, through which the fly eventually emerges in about six weeks.

Structure of the Proboscis.—The cutting mechanism of the proboscis is formed by the labella, the inner surface of which is provided with a number of fairly

* *Gl. palpalis* feeds principally on crocodiles (Koch).

coarse teeth and a series of file-like plates. These are capable of being completely everted and consequently, when the proboscis is rotated as a whole, would tear their way through the skin. The proboscis proper consists of three parts (1) the dorsal labrum, (2) the ventral labium, which partly embraces the labrum with a series of inter-locking teeth, and (3) the hypopharynx, which lies in a groove in the labium. The hypopharynx has two small lateral appendages, and the tips of the labrum are in apposition with these and keep it in its groove. Owing to the fusion of the labrum in the bulb of the proboscis, no independent longitudinal movements of these parts is possible. (Fig. 126A).

CLASSIFICATION OF SPECIES OF GLOSSINA

(A) Hind tarsi entirely dark.

(a) Abdominal segments with sharply defined pale hind borders. Second segment has a conspicuous square or oblong pale area in the centre.

1. *Gl. tachinoides* (= *Gl. decorsei*).—The smallest tsetse fly, 8 mm. excluding proboscis. ♂ smaller. In the ♀ the tarsi are basally somewhat pale.

(aa) Abdominal segments with hind borders, if lighter, extremely narrow. Second segment has pale area triangular. Larger species than (a).

2. *Gl. palpalis*.—Darkest of all species of *Glossina*. Third joint of antenna dusky-brown to cinereous black.

2A. *Gl. palpalis*, v. *wellmani* (= *Gl. bocagei*).—Distinguished from the type by its having the frontal stripe pale ochraceous; thoracic markings much reduced, so that the thorax in a well-preserved specimen appears spotted, the antero-lateral markings taking the form of spots or blotches; the spot immediately behind the

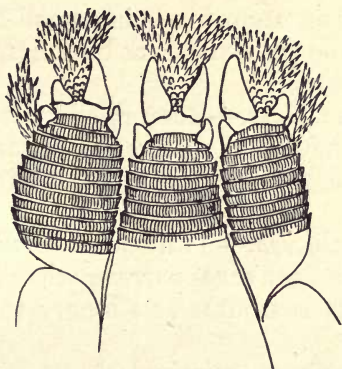
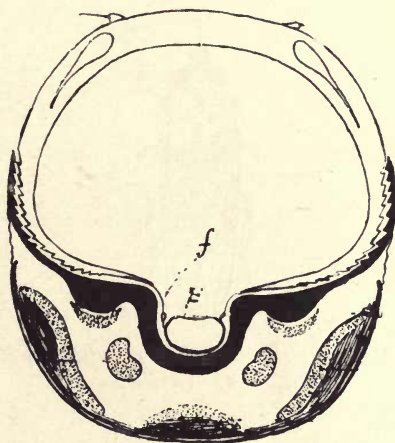


Fig. 126A.

- (1). *Glossina palpalis*. Inner aspect of inner wall of labellum shewing the cutting apparatus; this is everted during the act of biting



- (2). *Glossina palpalis*. Transverse (schematic) section of the proboscis just in front of the bulb. *e* = hypopharynx, *f* = the membranous portion of the labrum which is closely applied to the 'horns' of the hypopharynx. Above 'f' the interlocking teeth of the labrum and labium.

(After Stephens and Newstead)

humeral callus on each side being small ovoid or nearly circular; femora pale, the dark blotches much reduced (Austen).

3. *Gl. maculata*.—Resembles a dark *Gl. palpalis*, but differs in having the posterior surface of the head, thorax, pleurae, and femora, mottled with conspicuous black spots.

4. *Gl. pallicera*.—Third joint of antenna orange-buff. Front in both sexes *narrower* than in *Gl. palpalis*. In ♂ the arista is stouter and longer than that in *Gl. palpalis*.

(AA) Hind tarsi not entirely dark.

(a) *Small species*, length is rarely ten-and-a-half mm.

(β) Last two joints of front and middle tarsi have sharply defined dark-brown tips.



Fig. 127. *Glossina*, shewing scissors position of Wings when at rest, $\times 2$. (After Austen)

5. *Gl. morsitans*.—(1) Smaller than *G. longipalpis*. (2) Head narrower. (3) Front paler and wider. (4) Eyes in ♂ and ♀ distinctly converging towards vertex. (5) Abdominal bands less deep, pale hind margins of segments therefore deeper. (6) Hypo-

pygium in ♂ larger, paler, somewhat more oval in outline, and clothed with fewer hairs. (7) Tip of ♂ abdomen less hairy laterally. (8) Bristles on sixth segment in ♂ stouter and more conspicuous than in *longipalpis*.

6. *Gl. longipalpis*.

(ββ) Last two joints of front and middle tarsi entirely pale.

7. *Gl. pallidipes*.

(αα) *Large species*. Length at least ten-and-a-half mm. (in this respect they contrast markedly with the other small species).

8. *Gl. longipennis*.—(1) Thorax with four sharply defined dark-brown oval spots. (2) Ocellar spot, dark-brown, *very conspicuous* compared with the body. (3) Proboscis shorter than in *G. fusca*, and *relatively* shorter, compared with the body, than in any other species. (4) In both sexes the *front* is broader than in *Gl. fusca*.

9. *Gl. fusca*.—Thorax without spots.

2. *Sarcophagidae*.

Not blood-sucking. Arista feathery at the base, bare at the tip. Large flies, about 14 millimetres long.

Genus *Sarcophaga*.—Elongated thorax, three black bands, abdomen spotted. Third segment of antenna three times the second segment.

S. carnaria, *S. magnifica*, and *S. ruficornis* (India), give rise to terrible forms of myiasis in man and animals.

3. *Oestridae* (Bot. (=Larva) Flies).

Not blood-sucking. Large flies. Proboscis rudimentary. Antenna very short. Arista segmented. Flight humming.

(a) Genus *Gastrophilus*, e.g., *G. equi*. The white eggs can be easily seen on the horse's hair. The larvae

are swallowed and they attach themselves to the mucosa of the stomach.

(b) Genus *Hypoderma*, e.g., *H. lineata*. Larvae produce ox warbles (=tumours) in the ox.

(c) Genus *Oestrus*, e.g., *O. ovis*. Larvae in the respiratory passages of the sheep.

(d) Genus *Cephalomyia*, e.g. *C. maculata*. In the camel.

(e) Genus *Cephenomyia*, e.g., *C. rufibarbis*. In red deer. Scotland.

(f) Genus *Dermatobia*, e.g., *D. cyaniventris*. Larva is the 'ver macaque' (America), producing myiasis in man and cattle.

(g) Genus *Cordylobia*, e.g., *C. anthropophaga*. Larva is the 'ver de Cayor' (Senegal), producing myiasis in man.

Myiasis is common in Africa and in the tropics, but the larvae have been identified in but few instances as yet.

The fourth group, the Pupipara (to which *Glossina* also belongs, from the point of view of its life history), comprises :—

1. *Hippoboscidae* (spider flies).

They run rapidly over the body, hiding in hair or feathers. Head circular. No distinct neck. Clypeus distinct, separated from the head by a curved suture. Antennae lie in cavities in its anterior angle. Antennae: one segment with or without a style (arista). Palpi absent. Abdomen leathery, capable of much distension in ♀. Tarsi: fifth segment longest with two or three claws. Empodia (*between* the claws) distinct. Wings large, or mere strips, or absent.

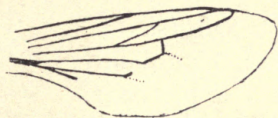
(a) Genus *Hippobosca*.—Wings large, obtuse. No

ocelli ; arista nude ; legs long and extended. Claws bidentate.

H. equini.—Runs rapidly over the body ; is the [New] forest fly of England.

H. camelina.—Attacks camels in Egypt.

H. rufipes.—Transmits *T. theileri* (?).



Wing of *Hippobosca rufipes*.

Fig. 128

(b) Genus *Melophagus*.—Wings extremely minute. Eyes small. No arista on antennae. Claws bidentate.

M. ovis is the sheep 'tick.' Four millimetres long.

(c) Genus *Ornithomyia*.—Wings large. Four millimetres long.

O. aricularia.—Occurs on birds.

(d) Genus *Lipoptena*, e.g., *L. cervi* on the red deer.

(e) Genus *Stenopteryx*, e.g., *S. hirundinis* of the swallow.

2. *Nycteribiidae*.

Found on bats. They have no wings.

CHAPTER XXXIV

FIXATIVES*

1. *Flemming's Solution*.—

Chromic acid, 1 per cent.	-	15 vols.
Osmic acid, 2 per cent.	-	4 vols.
Glacial acetic acid	-	1 vol.

Mix in the above proportions before use. Use *very small* pieces of tissue. Fixation is complete in about twenty-four hours. Wash well in water, and transfer gradually to eighty per cent. alcohol.

2. *Sublimate-alcohol*.—

Saturated watery solution of corrosive

sublimate	-	-	-	-	2 parts.
Absolute alcohol	-	-	-	-	1 part.

Fix small pieces for two or three hours more or less according to size. Transfer to eighty per cent. alcohol. Wash out the sublimate from the sections with Lugol's solution. Faeces containing *Amoebae* can be preserved by shaking up in this fixative and preserving in tubes.

3. *Acetic Alcohol*.—

Glacial acetic acid	-	-	-	1 part.
Absolute alcohol	-	-	-	3 parts.

Penetrates and fixes rapidly, according to the size of the tissue. It should be used where chitinous structures exist. Wash out the acid in alcohol.

*For *diagnostic* purposes dried smear preparations are of great value, but for studying the parasite in its relation to the tissues, *e.g.* in *Leishmania donovani* or *Histoplasma capsulata*, or in malaria, it is necessary to cut sections, and for this purpose the tissues must have been carefully prepared previously.

4. *Acetic Alcohol Sublimate*.—

Absolute alcohol	-	-	-	1 part.
------------------	---	---	---	---------

Glacial acetic acid	-	-	-	1 part.
---------------------	---	---	---	---------

Chloroform	-	-	-	1 part.
------------	---	---	---	---------

Sublimate, to saturation.

An excellent fixative for general purposes. Fix for one hour or several, according to size of tissue. Wash out the acid in alcohol.

5. *Formalin* (forty per cent. solution of formaldehyde).—Use ten per cent. solution in water or normal salt. Small pieces are fixed in an hour or so. Transfer to alcohol.

6. *Alcohol*.—Is a fixative and dehydrating medium, and should only be used alone in an emergency. The tissues, in small pieces, may be placed directly in methylated spirit (ninety per cent. alcohol), or absolute alcohol (ninety-eight per cent. alcohol). Change the alcohol a few times. After hardening, if the specimens are not to be imbedded immediately, transfer to alcohol of about eighty per cent. for preserving.

Rectified spirit of the British Pharmacopœia, is equal to eighty-four per cent. alcohol.

Methylated spirit, containing *wood* naphtha, is equal to ninety per cent. alcohol.

Ordinary methylated spirit contains mineral naphtha, and should not be used.

For practical purposes the dilution of alcohols is sufficiently accurately made by means of the diluting formula (p. 411).

7. *Zenker's Fluid*.—

Potassium bichromate	-	2.5 grammes.
----------------------	---	--------------

Sodium sulphate	-	1.0 gramme.
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Corrosive sublimate	-	5.0 grammes.
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Water	-	-	-	100.0 grammes.
-------	---	---	---	----------------

This is the stock solution. When about to use,

add one part of glacial acetic acid to twenty parts of stock. Fix until the tissue is opaque throughout (one or more hours). Wash in water till washings colourless and free from acid (an hour more or less). Preserve in eighty per cent. alcohol. This is an excellent general fixative.

N.B.—The sublimate is best removed from the sections before staining by Lugol's solution.

8. *Lugol's Solution.*—

Iodine	-	-	-	-	-	1'0 gr.
Potassium iodide	-	-	-	-	-	2'0 gr.
Water	-	-	-	-	-	100'0

TREATMENT AND PRESERVATION OF FIXED TISSUES

1. The best method, if possible, is after washing out the fixative to imbed the tissue at once or after some days in paraffin (p. 46). Pour the paraffin out into paper boats, small match boxes, etc. The tissues will then keep indefinitely.

2. The next best method is after washing and dehydrating to pass into cedar wood oil and preserve in the oil.

3. If neither of these methods is possible, then after washing (and passing through increasing strengths of alcohol, if required) to preserve in rectified spirit, or eighty-five per cent. alcohol or methylated spirit. The staining of tissues is somewhat imperfect after long preservation in alcohol.

WASHING THE FIXED TISSUES

If the fixative used has been an alcoholic one, then wash out in absolute alcohol (or spirit). (This, as will be seen, saves a good deal of subsequent trouble.) If the fixative has been a watery one, then it is

necessary to dehydrate by passing through successive strengths of alcohol, as the shrinkage would be great if the tissue were passed direct from water to absolute alcohol. This may be effected in the following way:—To 9 c.c. of water containing the washed tissue add 1 c.c. of absolute alcohol. The strength of alcohol is now ten per cent. Leave for ten minutes. Take out 5 c.c. and add 1 c.c. of alcohol, the strength is now twenty-five per cent. Leave for ten minutes. Take out 2 c.c. and replace by 1 c.c. of alcohol; the strength is now forty per cent. Leave for ten minutes. Continue in this way to take out 2 c.c. and replace by 1 c.c. of absolute alcohol. It will be seen that the process takes an hour. If the tissue is not going to be imbedded stop at eighty-five per cent. Otherwise at this stage place in absolute alcohol ready for passing through cedar wood oil.

N.B.—This process is only necessary where *extreme* care is to be taken in the preservation of histological details, otherwise change from the water to (about) eighty-five per cent. alcohol directly and renew it once.

TABLE FOR PASSING THROUGH INCREASING STRENGTHS OF ALCOHOL

9 c.c. H ₂ O	+	1 c.c. 100% alcohol	=	10 % alcohol.
5 c.c. 10 %	+	1 c.c. „ „	=	25 „
4 c.c. 25	+	1 c.c. „ „	=	40 „
3 c.c. 40	+	1 c.c. „ „	=	55 „
2 c.c. 55	+	1 c.c. „ „	=	70 „
1 c.c. 70	+	1 c.c. „ „	=	85 „

Any convenient multiple of these quantities may, of course, be used.

STAINS, ETC.

1. *Romanowsky Stains*.—*Vide* p. 21. The best stain for parasites in the blood, etc.

2. *Haematein*.—

Haematein (<i>puriss.</i>)	-	-	0.4 gr.
Alum	-	-	5.0 gr.
Glycerin	-	-	30.0 c.c.
Water	-	-	70.0 c.c.

Rub up the haematein with a little glycerin, and add the other ingredients. The stain is ready for use at once, and keeps well.

3. *Methylene Blue*.—For staining blood or tissues, use a quarter per cent. watery solution of pure methylene blue.

4. *Eosin*.—This is really Brom-eosin, *i.e.*, yellowish or water-soluble eosin. For blood work and also for tissues is best used in the form of a half per cent. solution in seventy per cent. alcohol.

Eosin*	-	-	0.5 grm.
Absolute alcohol	-	-	70.0 c.c.
Water	-	-	30.0 c.c.

5. *Ehrlich's Triacid*.—Take H_2O , 45 c.c.; glycerine, 10 c.c.; alcohol, ninety per cent., 25 c.c.; and add in the following order: acid fuchsin, 3 grm.; orange G, 2 grm.; methyl green, 1 grm. Stains typically and keeps well.

Iron Reaction (Haemosiderin) in Malarial Tissues.—

(1) Treat the sections after removing the paraffin with two per cent. watery solution of potassium ferrocyanide for from five to twenty minutes.

(2) Acid alcohol (HCl 1 part, seventy per cent. alcohol 100 parts) five to ten minutes.

(3) Wash in water.

(4) Counterstain with alum carmine.

To Mount Specimens in a Fluid Medium.—Remove all fluid from the edge of the coverglass. Seal with plenty of melted glycerine jelly. Examine next day to

* Use 'pure French' eosin or a spirit-soluble eosin.

see that air is not entering. When the jelly has set, it is advisable to paint over with a layer of some cement or varnish.

Glycerine-Alcohol.—Make seventy per cent. alcohol. Then make up five, ten, and twenty per cent. solutions of glycerine respectively in this.

To Mount Delicate Objects.—Fix in the five per cent. solution, heated to about 70° C., and then transfer gradually (at intervals of a day) from one solution to another. Allow the twenty per cent. solution to evaporate *slowly* (in a dry climate) or near the fire in a moist atmosphere. When the object is permeated, remove excess of glycerine and mount in glycerine jelly. This method is suitable for fixing and mounting small worms, *e.g.*, ankylostomes, etc., which would become too transparent in balsam.

Chlorine for Bleaching.—Place a little chlorate of potash in a test tube. Add some hydrochloric acid, and then some water. Place the object (fly, section of madura fungus, etc.) in, until sufficiently bleached. Wash out *thoroughly*.

PREPARING AND STAINING WET FILMS*

1. Expose a slide for two minutes to the vapour of—

Osmic acid, 1% - - - 1 c.c.

Glacial acetic acid - - - 4 drops.

2. Take up a drop of the blood on the slide and expose again to the vapour for thirty seconds.

3. Spread the film and before it can dry expose again to the vapour for fifteen to thirty seconds.

4. Absolute alcohol, ten minutes.

*These methods are employed where it is desired to avoid the distortion of structures which arises from drying films. The films are never allowed to become dry at any stage of the process.

5. Wash for one minute in—

Water	-	-	-	-	-	50 c.c.
Pot. permanganate,	1 % solution					2 drops.

6. Wash in water five minutes.

7. Stain with Romanowsky.

For Breinl's method, which is more complicated,
vide Annals of Tropical Medicine and Parasitology,
Vol. I, No. 3.

TO MEASURE OBJECTS

Method I.—It is necessary to have (a) a scale which is put into the eyepiece, and (b) a slide with a millimetre (divided into hundredths) scale ruled on it. Unscrew the top of the eyepiece and place scale (a) inside, place the top on, and by holding the eyepiece up to the eye see if the scale is in focus; if not, shift the diaphragm on which it rests up or down until it is so. Now focus scale (b) placed on the stage. Both scales will now be seen at the same time. Proceed then to find out how many divisions of the stage scale (b) one hundred, or fifty (or any smaller number) divisions of the eyepiece scale (a) cover. Let us suppose we find that forty divisions of the scale (a) cover eight divisions, *i.e.*, $\frac{8}{100}$ mm., *i.e.*, 80μ . Therefore one division of the scale (a) is equal to 2μ . Then knowing this—leaving the eyepiece scale in position and removing the stage micrometer—let us now measure a blood cell, and let us suppose we find it covers (using the same lens and eyepiece as before) three divisions. As we know that one division measures 2μ , the blood cell measures 6μ . Having once determined the value of the eyepiece scale for each combination of lenses and a definite tube length (160 mm.), we require scale (b) no longer. We simply place the scale (a) in the eyepiece, determine

how many divisions (*e.g.*, two) any body (*e.g.* a blood cell) covers, and multiply by 'the value' of each eyepiece scale for this combination of lenses. This method is more accurate than the following.

Method II.—It is necessary to have a camera lucida (the upright pattern is most convenient). Adjust the light of the mirror and the diaphragms of the camera lucida so that the object and the tip of the drawing pencil are both clearly seen. Mark out the required dimensions of the object, *e.g.*, length and breadth, on the paper placed at the right-hand side of the microscope, using the right eye for observing. Now place a millimetre scale divided into hundredths on the stage instead of the object. Draw the scale on the paper just below the object. Read off directly the size of the object in millimetres. The smallest amount that can be measured is $\frac{1}{100}$ mm. = 10μ .

NOTE.—By measuring the magnified size as drawn and dividing this by the actual known size of the stage micrometer the magnification of the microscope is given.

WEIGHTS AND MEASURES, ETC.

1. *Conversion from one Temperature Scale to another.*—

$$\frac{C}{5} = \frac{F - 32}{9}$$

Thus to convert 100° F. to centigrade—

$$\frac{100 - 32}{9} = \frac{C}{5} \therefore C = 37.7^{\circ}$$

2. *Formula for Dilution of Solutions.*—The number of parts required to dilute from one part of a solution of strength x per cent. to another strength y per cent.

$$\text{is } \frac{x}{y} - 1.$$

Thus, to dilute a solution from twenty per cent. to five per cent., add to each volume of solution

$$\frac{20}{5} - 1 = 3 \text{ of the diluting fluid.}$$

3. *Approximate Values.*—

1 cubic centimetre = 17 minims. 1 minim = .059 c.c.

100 cubic centimetres = 3 ounces, 4 drachms, 20 minims.

1 drachm = 3.55 c.c.

1000 cubic centimetres = 1.76 pints. 1 fluid ounce = 28.4 c.c.

4 litres = 7 pints. 1 pint = 568 c.c.

1 gramme = $15\frac{2}{5}$ ths grains (avoirdupois).

1 grain (avoirdupois) = .0648 gramme.

1 kilogramme = 2 pounds, 3 ounces, 119.8 grains (avoirdupois). 1 drachm = 1.77 grammes.

5 kilogrammes = 11 pounds. 1 oz. = 28.35 grammes.

1 grain apothecaries' = .0648 gramme.

1 drachm apothecaries' $\bar{3}$ = 3.88 grammes.

1 ounce apothecaries' $\bar{3}$ = 31.1 grammes.

15.4 grains apothecaries' = 1 gramme.

4. 1 millimetre = .039 ($\frac{1}{25}$) inch. 1 inch = 2.5 centimetres.

1 centimetre = .39 ($\frac{2}{5}$) inch. $\frac{1}{1000}$ inch = .0253 millimetre.

1 metre = 3.28 feet. $\frac{1}{100}$ inch = $\frac{1}{4}$ millimetre. $\frac{1}{1000}$ millimetre (μ) = .00004 inch.

5μ = $\frac{1}{5000}$ inch. $\frac{1}{4}\mu$ = $\frac{1}{10000}$ inch.

5. $\pi = 3.14159$.

Circumference of a circle = $2\pi r = \pi d$.

Area of circle = πr^2 .

STAINS, ETC.

Romanowsky.—

(a) Pure medical methylene blue.*

(b) Carbonate of soda.*

(c) Eosin* yellowish (water soluble), *e.g.*, B.A.†

This is the best form of Romanowsky for use in the tropics (p. 21).

Leishman.*—(= .015 gramme). Dissolve the *finely powdered* stain in 10 c.c. of absolutely pure methyl alcohol (got in tubes).

Giemsa.—Ready made (p. 23).

Jenner.—Ready made (p. 9).

Haematein (purissimus).

Fuchsin (basic).

Fuchsin (acid).

Orange G.

Methyl green.

Absolute alcohol.

Cedar wood oil.

Canada balsam (special acid free).

Xylol.

Paraffin wax (melting points 50° C., and 60° C.).

Superheated paraffin (Count Spee's).

Borax carmine. Ready made.

Glycerine.

Methylated spirit free from mineral 'naphtha.'

Egg-albumen glycerine.

Alum.

Eosin (1) B.A., (2) pure French.

* In soloids, Burroughs, Wellcome & Co.

† B.A., A.G., eosin extra, etc., are simply the various brands of various firms.

APPARATUS

1. An English microscope, large stand, with oil immersion $\frac{1}{12}$.
2. A mechanical stage.
3. Browning's pocket spectroscope.
4. Haemocytometer, Thoma-Zeiss.
5. Türk's Leucocyte counter.
6. Haemoglobinometer Tallqvist.
7. Sahli's haemoglobinometer.
8. Canada balsam bottle.
9. Pocket lens for mosquito and fly work.
10. Silver pins No. 20.
11. Fine Bristol board (cardboard).
12. Specimen tubes corked.
13. Glass slides 3×1 .
14. Coverglasses No. 1 $\times \frac{3}{4}$ in., $\frac{1}{2}$ oz.
15. Straight surgical needles.
16. Stoppered jars for fixing and staining
 $13 \times 7\frac{1}{2}$ centimetres.
17. Porcelain dishes, square, flat, 4×4 inches.
18. Graduated measures, 100 c.c. and 10 c.c.
19. 'Primus' paraffin burner.
20. Drop bottles for xylol, etc.
21. Petri dishes.
22. Slide boxes.
23. Camera lucida for drawing.
24. Stage and ocular micrometers for measuring.

LITERATURE

The Microtometist's Vade-mecum. Lee. Sixth edition.

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